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On the cover

Picture of delegates at the BISMiS 2018 International Conference

The Bulletin of BISMiS

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Bulletin Editorial

Paul A. Lawson

This issue of the Bulletin is globally diverse with articles from authors from the UK, US, Pakistan, India and South Africa. In Vol 7 part 1, we had a report from Kamlesh Janjid on the 3rd meeting of Bergey's International Society for Microbial Systematics in Pune, India, likewise in this issue Fanus Venter brings us a report from the 4th meeting hosted by the University of Pretoria. I can personally attest what a wonderful meeting this was with very active sessions and discussions in the spectacular setting of South Africa.

The second article is part 2 of Jim Staley's autobiography. In the last issue, Jim left us with his introduction to Marine microbiology and this is where his engrossing story continues. His journey is truly inspiring not only to the younger members of our community but also to many of us who know Jim yet were unaware of the details of his wonderful life and all his contributions to microbial systematics. Again, I have received many positive comments from our members on the motivational nature of the article. Such comments and feedback reinforce the interest in this type of article and is the goal of these autobiographical articles to allow insights into the individuals behind the familiar names.

The third and fourth articles focus on Culture Collections that represent invaluable global resources; the first is by Barry Holmes who provides a comprehensive review of the National Collection of Type Cultures (NCTC) one of the world's first and foremost collections that became a model for many that were later established. The second is by Iftikhar Ahmed Saira Abbas and Hamza Tariq providing information on the Culture Collections located in Pakistan and describes some of the history and challenges encountered in establishing these important resources for the scientific community. We close with two articles that provide updates and reviews on two groups of organisms; the first focuses on halophilic archea from saline environments in India by Pradnya P. Kanekar and Snehal O. Kulkarni, the second is by Imen Nouioui and her "love affair" with the genus *Frankia*.

Finally, as usual I wish to encourage readers or solicit friends and colleagues to submit articles for publication in future issues of the Bulletin, from original articles, reviews, and autobiographies. I especially ask students and postdoctoral scientists to submit contributions of their experiences (good and bad!) that help mold their early careers.

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BISMiS 2018, the Microbial Systematics Indaba

Fanus Venter

The University of Pretoria had the privilege of hosting the 4th BISMiS meeting in South Africa during April this year. The meeting of the *Bergey's International Society for Microbial Systematics* has become known as the foremost meeting for microbiologists sharing a passion for bacterial systematics and this conference lived up to this notion. The conference was attended by delegates from 14 different countries, representative of several regions of the world.

Figure 1. Delegates of BISMiS 2018

Having this meeting for the first time in Africa provided the opportunity to give the conference a true South Africa identity by organising it around the idea of an "indaba". An indaba, in the traditional African culture of Zulu and Xhosa speaking people, is a gathering where people get together to sort out the problems that affect them all. At these gatherings, everyone has a voice and an attempt is always made to find common ground and to collectively decide how to proceed. Formal and informal discussions on the future of bacterial systematics therefore formed an integral part of the conference. One such session dealt with the future direction of species descriptions and was facilitated by the editors of the three main journals in the field, i.e. Martha Trujillo (IJSEM), Iain Sutcliffe (Antonie van Leeuwenhoek) and Ramon Rossello-Mora (Systematic and Applied Microbiology). The conference was concluded with a final discussion "Bacterial systematics: What lies ahead?" lead by Iain Sutcliffe the current BISMiS president.

The scientific programme focused on various relevant aspects related to bacterial systematics. The opening address was given by Ramon Rossello-Mora from the Mediterranean Institute for Advanced Studies in Mallorca, Spain. In his presentation, "A need for taxonomists to take action before it's too late" Ramon discussed the urgent need to create a stable taxonomy with validated names for the uncultured bacteria. Other invited speakers included well-known taxonomists such as Barny Whitman from the University of Georgia (USA), Brian Hedlund from the University of Nevada Las Vegas (USA), Svetlana Dedysh, Winogradsky Institute of Microbiology, Moscow (Russia), Wilhelm de Beer from the University of Pretoria (South Africa) and Wen-Jun Li from the Sun Yat-Sen University, Guangzhou, (China).

Figure 2. Delegates enjoying the BISMiS 2018 Banquet
Foreground (L-R): Fanus Venter, Iain Sutcliffe, Martha Trujillo, Fred Rainey
Background (L-R): Paul de Vos, Vartul Sangal, Pierr Edm, Svetlana Dedysh, Ramon Rossello-Mora, Jongsik Chun

The Bergey's Manual Trust was one of the main sponsors of the conference. They supported Jongsik Chun from the Bioinformatics Institute at Seoul National University (Korea), to attend the meeting in order to receive the 2018 Bergey Award. Prof Chun also had the opportunity to reflect on the use of genomic data in bacterial taxonomy and metagenomics during the special award lecture. They also sponsored a number of travel awards for students and other young investigators to attend the meeting and used the opportunity to have a meeting of the Board of Trustees.

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Sponsorship was also received from IJSEM, Antonie van Leeuwenhoek and Systematic and Applied Microbiology. IJSEM sponsored three student prizes. Marike Palmer, University of Pretoria (South Africa) won the prize for the best postdoc oral presentation, Raul Riesco, University of Salamanca (Spain) for the best student oral presentation and Kgothatso Chauke, Agricultural Research Council, (South Africa) for the best poster (Figure 3).

Figure 3. Prize awardees (L-R): Raul Riesco, Martha Trujillo (Editor of IJSEM), Kgothatso Chauke, and Marike Palmer.

Figure 5. (L-R) Fred Rainey, Fanus Venter and Iain Sutcliffe enjoy after-dinner drinks and fine conversation

Delegates were treated to real African experiences such as the traditional braai (barbeque) and a drumming session which formed part of the welcoming function (Figure 4). In addition, delegates had plenty of opportunities to meet old friends and continue the discussion from the formal sessions (Figure 5 and 6). They also had a truly authentic African dining experience where they could feast on a variety of meat, including game at the Carnivore restaurant (Figure 7). Several of the delegates also joined the tours to the Sterfontein Caves (Figure 8), the Lion park (Figure 9) as well as the Lesedi Cultural Village (Figure 10) and Pilansberg nature reserve (Figure 11).

Figure 4. Delegates enjoying the drumming session that formed part of the welcoming function

Figure 6. Paul Lawson and Mike Goodfellow putting the microbial world to rights!

Figure 7. Food and good conversation at the Carnivore restaurant

Figure 8. Sterfontein Caves

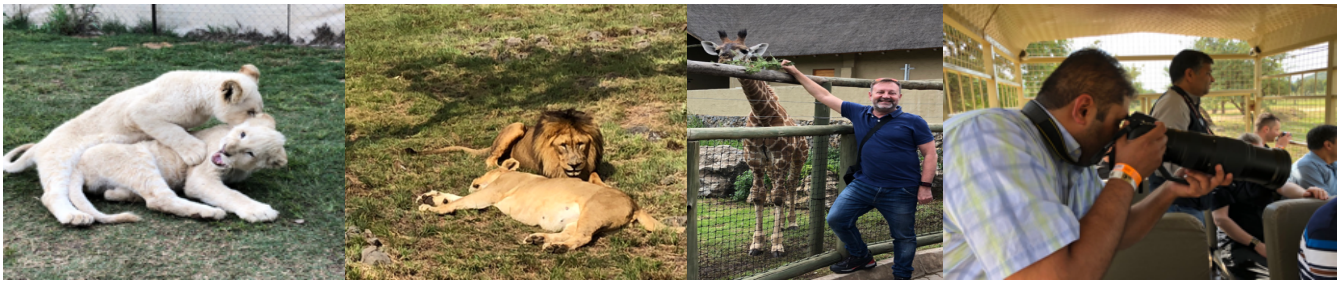

Figure 9. Lion park (L-R): cubs at play; adults chilling out; Ramon with a new friend!; Krithi Sankaranarayanan, now that's a big lens

Figure 10. Lesedi Cultural Village

Figure 11. Pilansberg Nature Reserve

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Seeking Truth in the Microbial Cosmos (*cont'd)*

James Staley

Marine Microbiology

I was first introduced to marine microbiology in North Carolina where I was invited to be an assistant on a short marine cruise on a Duke University boat with John Hobbie from North Carolina State, Professor Lawrence Pomeroy from U of Georgia and others who were comparing procedures for studying biological community biomass and activity - a wonderful experience.

Later Thomas Odum, a Professor in our Department at UNC in Chapel Hill was conducting a study of ponds that contained 50% sea water mixed with 50% wastewater in Morehead City on the Carolina coast. I set up the immersed microscope system to study the growth of algae in these lagoons and quickly realized that the algae in this system behaved entirely differently from the freshwater habitats in Michigan. They did not remain attached in one place on the glass slide surface! Clearly, I could not re-locate individual clones to study their development - the study had to be abandoned.

An early UW study entailed the use of microbial plating using 0.01% peptone to assess microbial diversity. Samples were spread plated from various habitats and morphological colony types were identified with a dissecting microscope. One sample was from an incoming tide at Deception Pass in Washington State. The results were surprising. Almost every colony type on the marine medium was different! It is the most diverse viable sample I have ever seen. In 2015 reports were published that Puget Sound's ocean influent was highly enriched in nutrients which helps explain the high diversity.

Whale microbiology and chitin degradation

Russell Herwig received his PhD from John Liston and became a postdoc. Russ and I decided to study the microbiota of baleen whales. Coming from Hungate's lab I knew something about the cattle forestomach fermentation. We hypothesized that, since whales are descended from land mammals and had similar digestive systems to ruminants, they might have a forestomach fermentation akin to cattle. That is how we came to study whales.

Our hypothesis was, if baleen whales were like ruminants, their fermentation products might be the same, in particular they may produce the volatile fatty acids (VFAs), acetic, propionic and butyric. The most daunting aspect of studying the intestinal microbiota of whales, is obtaining fresh samples. We contacted several labs that had stored samples from whale forestomachs. Having assembled a small collection of samples we subjected them to VFA analyses. Much to our delight, all the samples contained VFAs providing the first evidence for a forestomach fermentation (Herwig et al. 1984 AEM 47:421). Importantly, the substrate land ruminants use to produce VFAs is cellulose, comprised of glucose subunits, which is not in the diet of marine mammals. The marine counterpart is chitin, and its subunit is N-acetyl glucosamine. We wondered whether chitin was being degraded to form the VFAs by marine mammals that relied largely on krill, whose exoskeletons are largely chitin.

NSF Polar Programs supported this work. The best whale samples we obtained came from Iceland. Because our son, Greg, became ill during this period, Russ led the Iceland effort. Jay Stemmler, a technician, and Russ collected the freshest material possible, not only to analyze for chitin degradation, but also for forestomach acids. This approach alleviated concern about post-mortem fermentations. Fin whales were sampled shortly after the whales were 'landed' on ship. The chitin degradation work involved taking samples from several positions in the digestive tract, incubating them anaerobically and testing for chitin concentration over time. If they degraded chitin, we should see a decline in chitin concentration (chitin degradation could be due either to microbial or whale chitinases).

In Antarctica we used the same approach for testing chitin degradation in crab-eater seals. Penguins were captured and held in a container and fed controlled amounts of chitin (labeled with inert beads to begin and end the experiment) for a week before release.

The animal experiments gave similar results, i.e. about 25 % of the chitin was degraded through animal passage.

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The most likely explanation is that animals play little if any role in chitin degradation in the animal digestive tract but instead rely on the chitinolytic bacteria in their intestines. If the animals play a degradation role, it is likely minor when one considers the entire chitin pathway in the marine environment. Russ led the chitin degradation work in marine waters and sediments with Nancy Pellerin, a technician, Roar Irgens and James Maki (Herwig et al., 1988 *FEMS Micro Ecol* 45:21). Analyses we made of the chitin cycle illustrate (Staley and Herwig, 1993 In *Antarctic Microbiology* E. I. Friedmann, ed Wiley-Liss, Inc New York, pp 241-264) most chitin degra-dation occurs in sediments where chitinolytic bacteria reside.

In retrospect, the dominant role of bacteria in chitin degradation seems perfectly reasonable in light of the much later evolution of mammals and birds in comparison with zooplankton and krill with chitin exoskeletons. Like cellulolytic activities in the ruminant, the chitinolytic activities seem largely if not exclusively microbial. Regrettably, we lacked sufficient resources to identify and name the chitinolytic bacteria.

Polycyclic Aromatic Hydrocarbon (PAH) degradation and the Dilution to Extinction Most Probable Number (MPN) Procedure

For an extended period Russ and I collaborated with civil engineer professors John Ferguson, David Stensel and Stuart Strand. In the 1990's Professor Jody Deming directed a five year program on marine bioremediation in which we all participated. My lab developed enrichment cultures for marine polycyclic aromatic hydrocarbon(PAH) - degrading bacteria. Our approach was straight-forward: marine sediments were collected, mostly from Puget Sound, in particular from an EPA Superfund Site located in Winslow Harbor on Bainbridge Island contaminated by a defunct privately owned creosote wood treatment facility.

This combination of selective enrichment procedures with the quantification of specific groups of organisms assessed using MPNs (Dilution to Extinction MPN) became standard operating procedure in my lab. When important activities were discovered, the most numerous organisms were quantified by MPN from habitat samples. Enrichments with MPN dilutions retrieve the most numerous and environmentally significant organisms that may not dominate in undiluted enrichments. In MPN the highest dilutions that showed degradation were used to isolate, describe, name and place in culture collections the most numerous active organisms from an environment. *If ordinary enrichment techniques are used the predominant organism in an enrichment culture would be one favored in that enrichment, not the most numerous organism and important organism from the environment.*

After collection, sediments were diluted in a series of ten-fold dilutions in half-strength seawater medium amended with either naphthalene or phenanthrene, two common low MW PAH compounds. Enrichments were incubated at 15oC and examined daily for the telltale appearance of yellow to red-colored degradation intermediates that are signs of PAH degradation. The highest dilution enrichment cultures that were positive were streaked to isolate the responsible bacteria.

Sheryl Dykesterhouse was an MS student who led the first studies and two PhD students, Allison Geiselbrecht and Brian Hedlund continued work on this topic, which also included studies in the Anacortes area near the oil refineries. The result was the isolation of several previously unknown genera and species. The most remarkable example appeared in the highest dilutions of our PAH enrichments. We characterized and named the predominant novel genus and species *Cycloclasticus* ('ring-breaking cuss') *pugetii* after Peter Puget for whom Puget Sound was named (Dyksterhouse et al 1995 *IJSEM* 45:116).

Allison led the study of the incidence of PAH-degrading organisms from contaminated marine sediments near Winslow (Geiselbrecht et al 1996 *AEM* 62:3344). Allison and Brian also discovered and named other PAH degraders including a *Marinobacter* strain (Hedlund, Geiselbrecht, Staley 2001 *FEMS Micro Ltr* 9992:1) and *Vibrio cyclotrophicus* from Puget Sound (Hedlund, Staley 2001 *IJSEM* 51:61). An additional marine genus and species, *Neptunomonas naphthovorans* was also described (Hedlund et al 1999 *AEM* 65:251). Allison furthered our understanding of *Cycloclasticus pugetii* biogeography by isolating other strains from the Gulf of Mexico off the coast of Texas (Geiselbrecht et al. 1998 *AEM* 64:4703) before the British Petroleum (BP) oil disaster.

Subsequently others confirmed that *C. pugetii* and other *Cycloclasticus* species are the most numerous and dominant marine PAH degraders on a global scale (Staley 2010 Handbook of Hydrocarbon and Lipid Microbiology, K. N. Timmis Ed-in-Chief). I was also invited to Xianmen, China to give a seminar on *Cycloclasticus* and PAH degradation.

After the marine PAH-degradation program ended, we submitted a proposal to a new bioremediation initiative to continue our work. Regrettably, after submitting our full proposal, the panel decided they would not consider funding any marine proposals on HC degradation! This seemed especially odd because marine hydrocarbon spills pose some of the most significant problems in bioremediation whereas

contaminated soils are much better contained and were already well funded.

We were able to limp along with much-needed Sea Grant money for a time before our studies were deemed 'too basic' by a critical reviewer. This ended our efforts on marine bioremediation. I sadly shook my head in 2009 when the BP oilrig disaster occurred in the Gulf of Mexico. I knew then, as later reported, that *Cycloclasticus* spp would become predominant in the Gulf due to the release of all the uncontained PAHs during the spill. The result was unnecessary and caused incomprehensible damage to the marine biota and the livelihoods of millions of innocent civilians who lived on, near or from the Gulf's violated ecosystem which is still affected today.

Sea ice microbiology

My lab became involved in sea ice microbiology indirectly. The chitin degradation work led to the isolation of chitin-degrading bacteria from marine waters of the Antarctic Peninsula. Professor Roar Irgens, on sabbatical leave with me, had also studied and named gas vacuolate bacteria, suggested we look at our chitin plates to see if there were any chalky colonies indicative of gas vacuolate bacteria. Eureka - there were! This was most surprising as no one had previously reported cultures of gas vacuolate bacteria from the marine environment (Irgens, Suzuki, Staley 1989 *Curr Microbiol* 18:262).

Fig 7. Jim Staley obtaining ice core from McMurdo Sound near sea ice air strip.

We pondered why they were present in sea ice. Knowing that gas vacuolate bacteria are associated with gradients, I hypothesized they might stratify within the sea ice microbial community (SIMCO). Unfortunately, by then, the annual sea ice on the peninsula had melted. Therefore, we proposed to NSF Polar Programs to go to the main McMurdo base to sample the following year.

We were funded and returned to McMurdo the following year obtaining several cores from McMurdo Sound near the ice airstrip and also across the Sound at Marble Point (Fig. 7). The upshot was that gas vacuolate bacteria were found in all ice cores suggesting they use the vesicles to rise in the water column where they could freeze in the SIMCO (Gosink, Irgens, Staley, 1993 *FEMS Micro Ecol* 102:85; Gosink, Staley 1995 *AEM* 61:3486).

This discovery led me to hypothesize that the sea ice bacteria in the South Pole might be different from those at the North Pole because of the great separation in distance and low temperature of viability of these bacteria may support their independent evolution at the two poles (discussed in Evolution section).

Low temperature growth and psychrophile genomics

I became curious about the lowest growth temperature of the sea ice psychrophile we named *Psychromonas ingrahamii* (Auman et al 2006 *IJSEM* 56:1001). To determine this, we used a low temperature water bath set at -12 C. Full strength marine water broth was inoculated, incubated and observed daily to follow turbidity. Some test tubes froze early and no growth occurred in them. In the other replicates, turbidity was measured as growth proceeded. The doubling time calculated for several replicates was 240 hours (10 days).

Although this species might grow at a lower temperature, all tubes incubated at -15 C froze so our experiments could not be completed. Jennifer Breezee an undergraduate and Nate Cady a rotating graduate student aided with this work (Breezee, Cady, Staley 2004 *Micro Ecol* 47:300).

John Ingraham expressed an interest in having the genome of *P. ingrahamii* sequenced. Under Monica Riley's leadership (Riley et al. 2008 *BMC Genomics* 9: 210) I was delighted to help because Professor Riley taught me microbial genetics while I was at UC Davis - it was wonderful to pursue this with her. The results indicated that *P. ingrahamii* contains six classes of proteins, at least one more than other bacteria, their membranes may have a lower hydrophobicity with excess asparagines and other features that enhance psychrophily.

Black Sea Studies

Beginning about 2002, my lab collaborated with Professor James Murray in Oceanography. As a chemical oceanographer, Jim was interested in the chemical and microbial activities, particularly those in the nitrogen cycle in the suboxic zone of the Black Sea. He had two PhD students, Clara Fuchsman and John Kirkpatrick with whom we worked.

Two postdocs in my lab, Sujatha Srinivasan and Brian Oakley, led our studies. John and Brian were lead authors on a paper based on using PCR procedures to identify Planctomycetes in the Black Sea (Kirkpatrick et al 2006 *AEM* 72:3078). This phylum contains the anammox (anaerobic ammonia oxidation) bacteria which carry out this process known to occur in the Black Sea. Interestingly, our studies showed that Planctomycetes and related organisms occur throughout the water column even deeply into the sulfidic zone.

We later named a new un-isolated anammox *Candidatus* species "*Scalindua richardsii*" after Francis A. Richards, a UW chemical oceanographer who hypothesized the existence of anaerobic ammonium oxidation from chemical fluxes (Fuchsman et al. 2012 *FEMS Micro Ecol* 1-15). We also studied denitrification, nitrogen fixation, CO2 fixation, etc in a series of papers.

I was especially impressed by Clara's ability to relate the bacteriology to the chemical activities using techniques such as TRFLP and chemical isomers. Her work was regarded as the best example of interdisciplinary work in Chemical Oceanography by her PhD Committee.

Evolution

I have always been interested in investigating bacterial evolution, which was made possible by Carl Woese. His Tree of Life was constructed by analyzing the ssu rRNA sequences of representatives of all organisms. I had known Carl since I first corresponded with him in 1980 and provided him with samples of unusual bacteria for his studies. Later, Jerome Perry and I visited and interviewed him for the Saunders edition of our textbook Microbial Life where he was one of the featured micro-biologists. He was also a co-author on our paper describing *Polaribacter* as a new genus with four species, which were the first gas vacuolate members of the Bacteriodetes phylum (Gosink, Woese, Staley 1998 IJSB 48:223).

I was surprised during our last communications in late November, 2011, a year before Carl died, that he was totally unaware that Bergey's Trust had completely adopted his phylogenetic approach for bacterial classification. When I learned this, I immediately sent him a pdf file showing that the entire classification of the Bacteria and Archaea was based on 16S rRNA gene sequences from Domain to Genus. This made his day! In his last correspondence he stated: "You I trust to do it right! All the best for the holidays old friend. Carl"

Woese's incomparable research contributions provided bacteriologists with 16S rRNA sequence analyses and the prospect of using other gene and protein sequences to explore important and unanswered evolutionary questions about microbial biogeography, speciation and the origin of the Domains. The ability to study microbial evolution was one of the most exciting opportunities during the culmination of my career.

With the help of Evgeni Sokorenko and Roger Buick I initiated a graduate level course in Microbial Evolution, which Evgeni is still teaching as one of the most popular advanced courses in the Department of Microbiology.

Phylogenomic Species Concept and Universal Species Concept

As a taxonomist, I am interested in speciation, the evolutionary process whereby new species evolve from existing species and all species eventually become extinct. The ssu rRNA is too highly conserved to identify species. For species, the traditional method uses DNA – DNA hybridization (DDH) between two strains. An artificial cut-off of >70% for species was established by the work of John Johnson and Don Brenner, largely from the well-studied enteric bacteria.

Unfortunately, DDH is not an evolutionary method but the multilocus sequence analysis (MLSA) developed in Professor Brian Spratt's laboratory in London is. Using MLSA, the sequences of less highly conserved housekeeping genes can be concatenated to permit the phylogenetic identification of Bacteria and Archaea species and subspecies and is officially accepted by bacterial taxonomists (Stackebrandt et al 2002 *IJSEM* 52:1043).

I invited Brian to participate in an ASM symposium I organized. While there he suggested we propose a meeting of the Royal Society of London on the topic of microbial speciation. The meeting, organized with the help of Matthew Fisher, was an excellent opportunity for taxonomists and evolutionary microbiologists to discuss the application of MLSA for studies of microbial speciation, biogeography and biodiversity. At the meeting, I proposed the Phylogenomic Species Concept (Staley 2006 *Phil Trans R Soc B* 361:1899; Staley 2010 *Microbe* 4:361). By using 16S rRNA sequences for higher taxonomic levels, i.e. from the Domain to Genus and combining that with MLSA for the species and subspecies, the complete classification of Bacteria and Archaea can be determined phylogenetically.

The Phylogenomic Species Concept (PSC) can be applied not only to the Bacteria and Archaea, but to all organisms as a Universal Species Concept (Staley 2009 *Ind Micro Biotech* 36:1331 and Staley 2013 in The Species Problem – Ongoing Issues Igor Pavlinov ed, InTech Europe, Rijeka, Croatia). For example, a recent article in Science states that one cannot determine the species of birds (or yeast) by using a single gene's sequence (Zhang et al. 2014 *Science* 346:1311). Instead additional genes are necessary as with MLSA as stated by PSC.

Recently, we (Hedlund, Dodsworth, Staley 2015 *Syst App Micro* 38:231) explored considering the use of single cell and other genomic-PSC approaches for identification and tentative naming of un-isolated bacterial and archaeal species to expedite our understanding of microbial diversity without the need for cultivation.

Biogeography of Sea ice bacteria

The sea ice bacterial work led me to postulate that it would be important to know whether the same species of bacteria could be found in the North Polar SIMCO (Sea Ice Microbial Community) as the South Polar SIMCO. This hypothesis is based on the long distances between the North and South polar sea ice caps. Also, since we were studying extreme psychrophilic bacteria, it seemed reasonable it would be difficult, or perhaps impossible, for them to survive transfer across the equator by birds and other vectors because of warm equatorial temperatures. Each pole might serve as an independent place for evolutionary divergence. Understanding biogeography is especially important for Bacteria and Archaea because the traditional view was that the same species of bacteria will be found anywhere that environmental conditions are the same because of their rapid global dispersal.

The NSF ecology program funded us. We arranged to collect sea ice samples at Point Barrow as well as McMurdo, Antarctica. John Gosink studied this topic for his PhD. We collected sea ice samples from both poles and identified psychrophilic strains by fatty acid analyses and 16S rRNA gene sequences. At the time it was not known at what taxonomic level bi-polar bacterial endemism might occur and the bacterial species definition is based on an arbitrary cut-off point.

Several new genera and species of gas vacuolate bacteria were named including *Polaromonas vacuolata* (Irgens, Gosink, Staley 1996 *IJSB* 46:822) from the South Pole, *Octadecobacter arcticus* and *O. antarcticus* from each pole, respectively (Gosink, Herwig, Staley 1997 *SAB* 20:356) and *Polaribacter* with four species (Gosink, Woese, Staley 1998 *IJSB* 48:223) (Table 1) and summarized in our 'Poles Apart' paper (Staley, Gosink 1999 *Ann Rev Microbiol* 66:4104). Our results found the same genus, but not the same species at both poles. Interestingly, one of the polar genera we named, *Polaramonas*, has species indigenous in glaciers. A recent, more intensive study supports the view that different species of *Polaromonas* occur in the North and South polar glaciers (Gowar et al 2016 *Extremophiles* 20:403), which is completely consistent with our findings.

I was invited to present our results at UC Berkeley in the mid-1990s. Following my seminar I met with Professor Norman Pace who vociferously disapproved of even considering conducting studies on biogeography. I was shocked by his adamant denouncement. In my mind the question is not whether bacterial endemism exists, but at what taxonomic level might it occur? I reported we found the same genera at both poles, which I believe is important, but not the same species. Also I did not claim that my data were definitive.

In retrospect, it appears Norman Pace believes everything you need to know about evolution and taxonomy is revealed by 16S rRNA gene sequences. All microbial taxonomists know 16S does not typically identify a species, let alone subspecies and MLSA, as indicated previously, is officially accepted (cf Stackebrandt et al 2002 above) for identifying bacterial species and subspecies.

Rachel Whitaker et al (2003 *Science* 301:976) subsequently published a definitive paper showing endemism of "*Sulfolobus islandicus*" strains isolated from hot springs in North America, Iceland and the Kamchatka Peninsula in Russia. She noted in her paper that 16S was unable to distinguish among the 70+ strains she studied, but MLSA of less highly conserved genes did. More recently, we (Zuo, Hao, Staley 2013 *Ant van Leeuw* DOI 10.1007/s10482-013-0081-4) confirmed Rachel's findings that strains were subspecies using electronic DNA-DNA hybridization of several "*S. islandicus*" genomes.

Co-Speciation of *Simonsiella*

Professor Daisy Kuhn (daughter of chemist, Thomas S. Kuhn) conducted beautiful work on *Simonsiella*, a gliding bacterium that is a bacterial inhabitant of the oral cavity of mammals. Daisy, who was a PhD student at UC Davis before I arrived, assembled a collection of *Simonsiella* strains isolated from cats, dogs, sheep and humans. She suspected that they co-speciated with their mammalian hosts.

Brian Hedlund shared my curiosity, and we decided to test the hypothesis that co-speciation accounts for the evolution of separate species for each animal host. 16 strains were obtained from ATCC, four of each from the four host species.

Brian completed the 16S sequences, and, as predicted, each *Simonsiella* species had its separate cluster of strains with the host animals so their phylogenies mirrored that of their host. Thus, the cats and dogs, as carnivores, formed two subgroups of one branch, and the human and sheep strains were on separate branches of the mirror image (Staley 2006 *Phil Trans R Soc B* 361: 1899). This raises questions about the taxonomy of bacteria versus that of animals because the single genus *Simonsiella* co-evolves with different families of animal hosts.

However, when other oral bacteria were included in the Trees, we found a more mixed picture: strains of closely related bacteria especially *Neisseria* spp. were interspersed within the human, dog, cat and sheep strains (Hedlund, Staley 2002 *IJSEM* 52:1377).

We infer the most likely explanation is that *Neisseria* and related species must have obtained 16S sequences from lysed *Simonsiella* cells in the environment, but, in contrast, *Simonsiella* must have a mechanism to reject or degrade these foreign sequences; an interesting question that could be experimentally addressed.

The Planctomycetes, Verrucomicrobia, Chlamydia (PVC) Superphylum

Some of the most remarkable bacteria known are found in the PVC Superphylum (Wagner, Horn 2006 *Curr Opin Biotech* 17:241). Unfortunately, they are very poorly studied yet harbor unusual features and carry out activities not known elsewhere in nature. I was fortunate to have studied members of the PVC throughout my career, yet only more recently learning of their exciting importance to evolution.

Unwittingly, we isolated the first representative of the Verrucomicrobia in the 1960s (*Prosthecobacter fusiformis*) and also the first two species of the Planctomycetes in the 1970s (*Pirellula staleyi* and *Planctomyces maris*).

The Planctomycetes

While a PhD student at UC Davis, I isolated a pear-shaped budding bacterium from Putah Creek. A second isolate was obtained from Lake Lansing while at MSU. Because of other activities, I was not able to return to work on these organisms until UW in 1971. By then, only the Lake Lansing strain remained.

Following Arthur Henrici, who did not isolate it, I described it as a neotype strain of *Pasteuria ramosa* ala Metchnikoff (Staley 1973 *Can J Micro* 19:609). The subsequent isolation of a true *Pasteuria ramosa*, a pear-shaped bacterium containing endospores, indicated Henrici and I were mistaken. Nonetheless, the strain was clearly a member of the Planctomycetes, indeed, the first Planctomycete ever isolated in pure culture. That organism was subsequently named *Pirella staleyi* by Mortimer Starr and Jean Schmidt and later by Hirsch's lab, *Pirellula staleyi*, the name it still bears.

John Bauld, my second postdoc, isolated the second known strain of Planctomycetes - a stalked organism from Puget Sound we named *Planctomyces maris* (Bauld, Staley 1976 *J Gen Micro* 97:45). This organism has a true non-cellular stalk that mediates the attachment of the cell by the holdfast at its apical stalk tip. Stalks are fibrillar consisting of a fascicle of long fimbriae that extend from the pole of the cell with the holdfast at their tip (Fig 8). The organism's unique ecological feature is that it is tethered to a solid substratum by its holdfast and bounces about in micro-eddies-

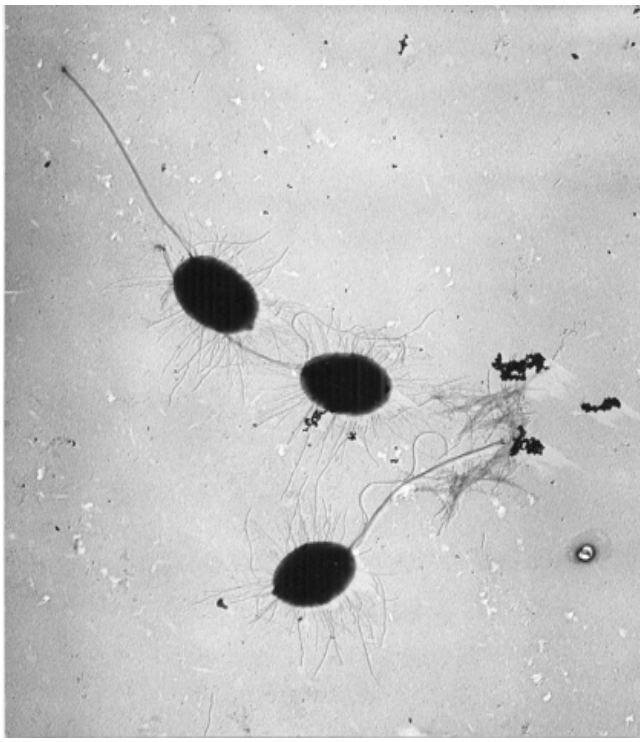

Fig 8. *Planctomyces maris*. Note fibrillar stalks, fimbrae, subpolar flagella and sole incipient bud on one cell.

Several labs I had contact with became centers for work on Planctomycetes and/or the polyprosthecate bacteria including professors Mort Starr at UC Davis with Jean Schmidt at ASU, Peter Hirsch who left MSU for a Professorship at Kiel, Germany, Professor Vik Skerman who headed the microbiology group at the University of Queensland when I was on sabbatical in Australia and Professor John Fuerst on sabbatical in my lab. Also, I sent strains of the prosthecate bacteria to Professor D. I. Nikitin's lab while still at UC Davis in 1996. Professors Lina Vasilyeva and George Zavarzin's group in the Soviet Union studied these organisms.

Those labs became international centers for the study of the Planctomycetes and Prosthecomicrobia including all facets of their biology. Likewise, we continued to study them throughout my career although not as a primary research focus.

Verrrucomicrobia, bacterial tubulin and evolutionary implications

Carl Woese became quite interested in the PVC Superphylum after publishing a paper with James Moulder's group at the Univeristy of Chicago showing that Chlamydia are members of the PVC group. In a 2008 E-mail, Carl told me he thought they should comprise a Kingdom.

The Verrucomicrobia

In about 2000, Carl Woese asked if I had any suggestions for bacteria to be sequenced at *Integrated Genomics* (IG), a startup in Chicago headed by Professor Robert Haselkorn. I provided one Verrucomicrobium (*Prosthecobacter dejongeii*) as well as a Planctomycetes soil strain (*Gemmata* Wa-1). Material was sent and sequences became available soon thereafter. I received a phone call from Ross Overbeek at IG when the *Prosthecobacter* sequence was complete. He said he was happy to do a search right then, if we had genes of interest. I called out to the lab members and Cheryl Jenkins, a post-doc from John Fuerst's lab said, "Ask him about tubulin." I knew immediately this was an excellent suggestion because Karl Schleifer's lab had preliminary evidence that an unisolated Verrucomicrobia species might have tubulin genes. So I asked Ross, who is not a microbiologist, and after a moment's delay he calmly responded, "Yes, they do." I responded incredulously, "Are you sure?" "Absolutely!" he replied. We were stunned.

With that, I immediately put Cheryl in charge of following up, and, as the data trickled in, it was one of the most gratifying discoveries of our lab. We first submitted the paper to *Science* for publication and were surprised they were not even interested in reviewing it - after all it was the first genetic evidence of tubulin genes in a prokaryote. I knew that Professor Lynn Margulis had published a paper in Science some time earlier in which she provided 'EM evidence for tubulin' in spirochetes that supported her hypothesis that spirochetes were the evolutionary forerunners of the eukaryotic flagellum which contains tubulin. But no spirochetes are known to have tubulin genes.

Carl Woese agreed to help with the review process at PNAS (Jenkins et al. 2002 *PNAS* 99:17049). Two tubulin genes were discovered that were homologs for α- and β-tubulin. We named them bacterial tubulin BtubA and BtubB representing their relatedness to α- and β-tubulin, respectively. Using PCR we verified that all four species of *Prosthecobacter* from our lab contained both tubulin genes. No other Verrucomicrobia or other bacterial or archaeal species is currently known to contain tubulin.

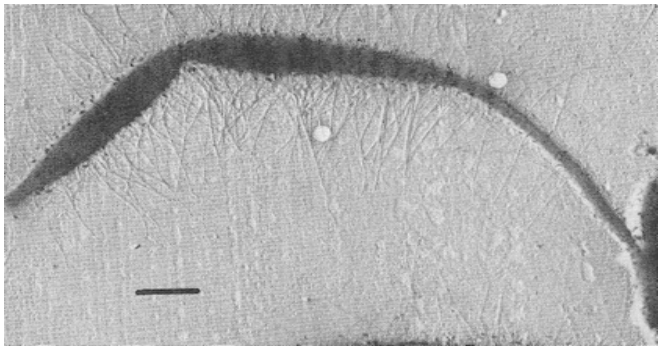

Fig 9. *Prosthecobacter fusiformis* bar is 1 micrometer - note prostheca and timbre

I knew Lynn since her sabbatical leave at UW in the early 1970s. She was a good colleague, and I later favorably reviewed her *Five Kingdom* book. Some time after our tubulin paper was published and shortly before her death, I received a phone call from her. We had a friendly exchange about a number of issues relating to photosynthetic bacteria she was studying. Incidentally, she mentioned how she had evidence from the fossil record of tubulin in a spirochete! It reminded me of a talk she presented at a FEMS meeting in Barcelona where she derided Carl Woese's evolutionary work on the Tree of Life. In retrospect, I believe she was reacting to confronting real scientific evidence that cast doubt on her *Five Kingdom* classification. I believe her talk was a tirade against the messenger because the message was too painful to accept. Paradigm switches are difficult for many scientists especially when their own contributions are at stake.

Nuclear Compartment Commonality (NuCom) Hypothesis

Regrettably, some scientists have recently attacked Woese's Three-Domain Tree of Life as being 'incorrect' because it does not fit their view of the evolution of the Eukarya from two prokaryotic lineages (a 'Prokaryotes First' hypothesis), the Bacteria and the Archaea. One group (Williams, Embley 2015 *Phil Trans R Soc Lond B Biol Sci* 370:20140318) replaced Carl's tree by a two-pronged 'stick figure' of two Domains Bacteria and Archaea, which give rise to the Eukarya, by invoking 'massive horizontal gene transfer'! In my view, one cannot discover the Eukarya if you are 'barking up the wrong Tree of Life'. Also, like Carl Woese, I believe it is highly unlikely that a bacterium and an archaeon could fuse together.

Further, it is scientifically impossible to test such a singular event – some philosophers regard an untestable hypothesis as being invalid scientifically for that reason (Richard A. Muller: *Now The Physics of Time* Norton 2016).

At the time we published the last edition of our textbook (2nd edition of Microbial Life with Sinauer) in 2007, I had doubts about having included a figure illustrating a fusion event for the evolution of the Eukaryotes.

About that time CG Kurland presented a seminar at UW. He espoused a "Eukaryotes First" hypothesis. Although I doubted that eukaryotes gave rise to the Bacteria and Archaea, Kurland's intriguing point to me was: it is simpler to produce a prokaryote from a eukaryote by reductive evolution, than a eukaryote by the fusion of two prokaryotes. I agree!

My NuCom hypothesis concerns the evolution of the Bacteria and Eukarya (Staley 2013 *Astrobiol Outreach* 1:105). I had one of those Eureka moments when I realized that the Planctomycetes and other members of the PVC Superphylum are nucleated. NuCom posits that the ancestors of the Bacteria and the Eukarya, which comprise disparate, independent branches of the TofL were both nucleated from the time DNA replication evolved.

According to NuCom, the Eukarya have always been nucleated and the ancestors of the Bacteria are the nucleated ancestors of the PVC Superphylum. Unfortunately, most microbiologists know little about the PVC Superphylum which is key to understanding the NuCom hypothesis. Support for NuCom comes from the fact that Planctomycetes are known to be the most ancient members of the Bacteria by two independent phylogenetic approaches, careful 16S and proteomic analyses (Brochier, Phillipe 2002 *Nature* 417:244; Jun et al 2010 *PNAS* 107:133). Other support for NuCom is that the cell (and hence nuclear) membranes of the Bacteria and Eukarya are essentially identical in composition, and, importantly, homologous enzymes synthesize them. Common Bacteria, such as *E. coli* are enucleate descendants of the PVC Superphylum that have lost their nucleus through reductive evolution. Other evidence of homologous proteins has been submitted (Staley, Fuerst. 2017, in press *Res in Micro*).

It is important to recognize that NuCom does not propose that the PVC gave rise to the Eukarya, a misguided view by some PVC adherents. The first genomics paper published on two PVC species *Prosthecobacter dejongei* and *Gemmata* Wa1-1 (Staley, Bozek, Jenkins 2005 *FEMS Micro Ltrs* 243: 9) concluded that it was doubtful that the PVC gave rise to the Eukarya but they shared important ancient proteins such as tubulin from LUCA (Last Universal Common Ancestor) as explained by NuCom (see Staley, Fuerst paper above).

I believe there is more evidence to support NuCom than any other hypothesis about the origin of the Eukarya. However, without providing evidence to the contrary, Norm Pace and W. Ford Doolittle do not agree. In contrast, I believe my good friend, Carl Woese would. When confronted with evidence for NuCom, doubters are inexplicably unconvinced.

From an aesthetic viewpoint of Truth (J W McAllister *Beauty and Revolution in Science* 1996 Cornell University Press, Ithaca), NuCom provides the simplest, most beautiful explanation for the evolution of the Bacteria and the Eukarya. No one needs to explain how an Archaeon evolved tubulin from FtsZ or artubulin, why the ribosomes of Archaea and Bacteria are different from the Eukarya or how the Eukayotes evolved their PLFA ester-linked membranes from an Archaeon, or invoke 'massive horizontal gene transfer' of ancient proteins. And most importantly a fantastic fusion event is unnecessary.

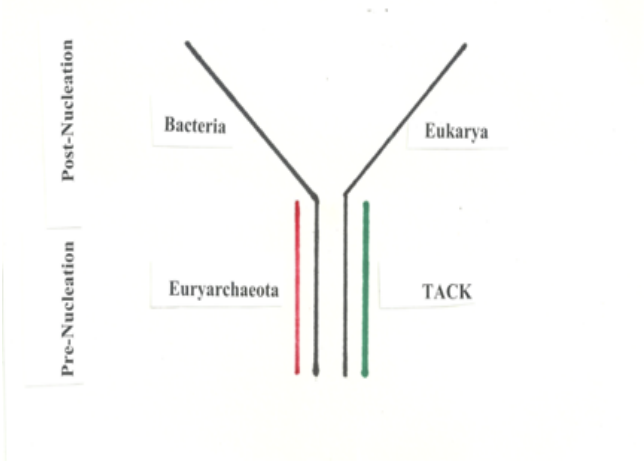

Figure 10. Tree of Life based on co-evolutionary processes. The two main lines of descent are the Bacteria and Eukarya. During the pre-cellular evolutionary stage the Euryarhaeaota evolved with the Bacteria and the Crenarchaeota (TACK) evolved with the Eukarya. After the Bacteria and Eukarya became nucleated this process ceased because their DNA was inaccessible.

In my view, those who believe in "Prokaryotes First" are constrained by a false paradigm. However, the NuCom hypothesis is a testable hypothesis and suggestions for testing it were included in Staley and Fuerst (2017). After I retired, I conducted theoretical work. One article proposes Domain Cell Theory on the evolution of the Bacteria, Archaea and Eukarya (*Open Biol.* 7: 170041 2017). Domain Cell Theory states that each of the Domains of life in Carl Woese's Tree of Life, i.e. the Bacteria, Archaea and Eukarya formed independent cellular lineages so that Bacteria—> Bacteria, Archaea—> Archaea and Eukarya—> Eukarya The Eukarya have always been nucleated and the earliest Bacteria (i.e. PVC phyla) were also nucleated (Figure 10).

In contrast the Archaea formed two independent cellular lineages, the Euryarchaeota and the Crenarchaeota, neither of which became nucleated. I have more recently collaborated with Gustavo Caetano-Annoles who introduced me to the importance of protein family-folding patterns and their evolutionary implications. We have two papers, one in press in *Bioessays* and another in preparation about the Tree of Life (Figure 10). This work confirms Domain Cell Theory.

Other Scientific Activities

Colleagues

In addition to my mentors, graduate students, technicians, postodocs and those on sabbatical leave who have been discussed, other students included Karen Junge, Ivy Suzuki, Gary Oertli, David Tison and Matt Stoecker. I have had the good fortune of supportive colleagues throughout my career. Many are at UW including John Sherris, Gene Nester, John Leigh, Charles Evans, Helen and Arthur Whiteley, Erling Ordal, Mary Lidstrom, Evgeni Sokurenko, Woody Sullivan, Jim Lara, Beth Traxler, Jim Champoux, Peter Greenberg, Steve Lory, Steve Moseley, Jody Deming, Roger Buick, Matt Parsek, Edward Haskins, Thomas Quinn, Beth Traxler, John Baross, Ron Merrill, Charles Evans, Neal Groman, Milt Gordon, Steve Lory, David Catling, Larry Corey, Julie Overbaugh, Tom Fritsche, David Stahl, Brian Lewis, Ludmilla Chistoserdova, Tom Lie, Frank and Ruth Harold, Kelly Hughes, Cary Hartman, Jim Murray, Al Devol, Marie Coyle, Clem Furlong (from UC Davis), Bob Waaland, Sharon Doty, Ben Hall, Joe Felsenstein, Claire-Horner Devine, Joseph Ammirati, Gordon Orians, Conway Leovy, Roger Buick, David Catling, Eric Cheney, Deborah Kelly, Don Brownlee, Steve Warren, Peter Ward, Woodruff Sullivan, Richard Smith, Dennis Kunkel, Rosanne Cattolico, Imre Friedmann, Joe Ammirati, Thomas and Yvette Edmonson, Karl Banse, John Adams, Gustavo Caetano-Annoles, Ramona Memmer, Janis Fulton, Dale Parkhurst, Dorothy Cramer, Mary Bicknell, Carol Laxson, Kendal Gray, Mark Chandler, Sarah Mears, Jane Halsey, Bonnie Hightower.

Other colleagues at UW are John Liston, Robert Wissmar, John Ferguson, Deborah Kelly, David Stensel, Eric Cheney, Rita Horner, Stuart Strand, ceramic engineers Ilhan Aksay (now at Princeton) and Mehmet Sarikaya with Tao Ren, and Tao's colleague Xian Ming Shi who hosted Sonja and me in Shanghai during the 2009 Exposition.

I also want to acknowledge Bergey Trustees and Associates: Robert Murray, Noel Krieg, John Holt, Marvin Bryant, Richard Castenholz, David Boone, Don Brenner, William Whitman, Fred Rainey, Michael Goodfellow, Karl Schleifer, Norbert Pfennig, Peter Sneath, Arnold Raven, Martha Trujillo, Peter Kämpfer, Jan Ursing, Stan Williams, James Moulder, Hans Lautrop, Paul De Vos, Michael Goodfellow, Wolfgang Ludwig, Ken-Ichuro Suzuki, Karl Stetter. Later, Associate Editors were invited to join meetings while the taxa they had expertise in were being written. I know many others in systematics including Antonio Ventosa, Dorothy Jones, Erko Stackebrandt, Hans Trüper, Paul Lawson, Rita Colwell, Aiden Parte, Cletus Kurtzman.

Other international colleagues include Tony Walsby, Heinz Schlesner, Thomas McMeekin, Ramon Rosello-Mora, Brian Spratt, John Bowman Huub J. M. Op den Camp, Essam Ghanem.

I enjoyed sabbatical leaves with Professors Vic Skerman and Kevin Marshall in Australia, Ralph Berger in Hawaii and Peter Hirsch with Heinz Schlesner in Germany. Also, I have many Australian, European and Asian friends and colleagues, including Professors John Fuerst, Lindsay Sly, Brian Spratt, Lise Øvreas, Bailin Hao, Aharon Oren,

A number of Postdocs in the lab included Russell Herwig, James Maki, Cheryl Jenkins, Sujatha Srinivasan, Brian Oakley, Tom Moench, Judith Bland, Robert Ward, Alex Semenov, Benjamin Yee.

Several sabbatical leave individuals also worked in the lab including John Fuerst, Don Johnstone, JoAnne Chee-Sanford, Roar Irgens, R. A. Sanford, Liv Fiksdal, Lina Vasilyeva, Jeanne Poindexter.

Bergey's and BISMiS

I became a Bergey's Manual Trustee in 1976. When I retired at the mandatory age of 70 in 2008, I was the longest serving member. For me, Bergey's was the ideal scientific organization with an honorable goal: working together to produce the most up-to-date, affordable and accurate listings of species and their characteristics along with a classification of the Bacteria and Archaea. Trustees collaborated in a congenial manner and worked with a supporting group of several hundred invaluable and dedicated contributors to produce a detailed description of species and the most recent classification. It was and remains a wonderful organization of scientists with an annual meeting held alternately in North America and Europe and more recently in Asia. John Liston was Chair of the Trust, and Professor Robert G. E. Murray followed him during the 1970s. Murray remained Chair until after the first edition of Bergey's Manual of Systematic Bacteriology (BMSB) was published.

John Holt was head of the editorial office when I joined and maintained that position until his retirement. John Holt and I were invited to the USSR to meet with George Zavarzin and others at the Institute of Microbiology at the time of *Glasnost* because Soviet microbiologists were interested in producing a Russian translation of Bergey's Manual of Determinative Bacteriology. While there, we met George Zavarzin, Lina Vasilyeva, D. I. Nikitin, and many other bacteriologists who were doing fascinating research at the Institute in Moscow as well as some at Moscow State University (E. N. Kondratieva) and in Leningrad (St. Petersburg) and also visited the Microbiology biotechnology center in Puschino led by Yuri Trotsenko where UW colleague, Ludmilla Chistoserdova studied.

Soon after I joined in 1976, John Holt and Bob Murray proposed that the Trust consider publishing a more comprehensive taxonomy that contained more historical and descriptive information about the bacteria and suggested calling this Bergey's Manual of Systematic Bacteriology (BMSB). This was unanimously approved by the Trustees. The first edition of BMSB was based on phenotypic properties of Bacteria except for the Archaea section led by Karl Stetter, which was based on 16S analyses.

Peter Sneath and I were particularly interested in producing a completely phylogenetic classification – at the time this consisted of computer print-outs of just a few pages from Carl Woese's work that I brought to the annual meetings. However, at the time of the second edition a complete truly phylogenetic classification based on 16S rRNA sequencing from Domain to Genus became possible (Fig 10). For the 2nd edition of BMSB, the phylogenetic work was admirably performed by Wolfgang Ludwig and Professor Karl Heinz Schleifer.

Figure 11. Trustees in London, Ontario 1990 L – R Back Bob Murray, Noel Krieg Front Don Brenner, Karl Schleifer, Jim Staley, John Holt, Norbert Pfennig, Peter Sneath

I became Chair of Bergey's Trust in 2000 amid a time of change after John Holt retired and George Garrity replaced him. The 2nd edition was behind schedule, and Michigan State University wanted to assert more control over the Trust. The upshot was that the Trust decided to move. The cordial move was made to the University of Georgia with William B. Whitman as head of the editorial office.

In addition to completing the 2nd edition, the other major accomplishment during my Chairmanship was the establishment of an international society for microbial taxonomists called Bergey's International Society for Microbial Systematics (BISMiS). We also initiated a newsletter, The Microbial Taxonomist, and the BISMiS Bulletin with biographies, reviews, opinion articles and invited autobiographies that I edited for two years.

The inaugural meeting of BISMiS was held in Beijing in 2011. This was a spectacular event led by the new Bergey Chair, Michael Goodfellow along with current Trustees and several hundred mostly Chinese and other Asian taxonomists in attendance.

I was invited by Ken-Ichuro Suzuki to deliver a 30th anniversary address at the Japanese Society of Microbial Syste-matics in Tokyo in 2012 where I discussed the history of Bergey's Trust and the phylogenomic species concept. I met Professor Emeritus Kazuo Komagata and invited and edited his autobiography for the Bulletin of BISMiS.

American Society for Microbiology (ASM)

ASM is another organization with great leadership that serves the larger community of microbiologists. I've been a member for over 50 years and served one term on the Council Policy Committee. I received the US Federation of Culture Collections J. Roger Porter Award, from ASM in 2008. My talk was entitled "Beyond 16S" which discussed speciation, MLSA analyses, biogeography and genomics. I have been honored to have two species named for me: *Pirellula staleyi* and *Polaribacter staleyi*.

Books

With Anna-Louise Reysenbach, I edited a book entitled *Biodiversity of Microbial Life: Foundation of Earth's Biosphere* (2002 John Wiley & Sons, New York).

In about 1990, Jerome Perry asked whether I would like to co-author a textbook for microbiology majors. After some consideration, I accepted. Our first edition was published with Saunders. Another two editions entitled *Microbial Life* were published by Sinauer Press, the last in 2007.

I have not kept up with current major's editions of textbooks that are available now. But, I must say, from what I know, I am concerned. Most disturbing to me is that there appears to be little if any mention of the PVC Superphylum in textbooks. This group of organisms is key to understanding the evolution of life, but many microbiology graduate students do not even know they exist! Is it any wonder that the 'Prokaryotes First' folks still have fertile ground to plant their myths about evolution? Also, there seems to be a tendency in texts to provide as many facts as possible. I believe much more needs to be done to put the facts in context with stories, particularly with respect to evolution. If 'dumbing down' occurs in advanced microbiology textbooks, it's a pity.

Astrobiology Doctoral Program

One of the most fun chapters in my career was the astrobiology program. It all began early in 1997 with a seminar program, *Planets and Life* originated by Woodruff 'Woody' Sullivan III in the UW Astronomy department and John Baross in Oceanography. They asked me to contribute to the seminar series by reviewing the recent paper on the evidence for life on Mars based on the recent discovery of the Allan Hills Martian meteorite from Antarctica. The seminar course involved faculty from several different disciplines including atmospheric sciences, aeronautical engineering, geology, biology, microbiology, oceanography, astronomy, genetics and biochemistry.

That fall, NSF put out a call for proposals to their new Integrative Graduate Education and Research Traineeship (IGERT) program. I asked Woody Sullivan whether he would be interested in applying to it. He said he was, but he was too busy at the time and suggested he would help if I took the lead on it. So, I reluctantly agreed and placed him and Conway Leovy as Co-PIs of the proposal. I wrote the proposal, circulated it to all the Co-PIs for input and submitted.

Never have I received such rave reviews. One panelist on the review committee commented: "What are they putting in their coffee in Seattle!" And so the program began in 1998. I was Chair of the AB for several years and was replaced by Woody Sullivan about the time I retired. The program is still active although the Department of Microbiology is less involved. Victoria Meadows is the current Chair.

I was invited to write a paper about the Astrobiology Program at UW that received input from all Co-PIs (Staley 2003 *Cur Op Biotech* 14: 347). We described astrobiology as a basic, integrative program for teaching science and engineering at all grade levels because astrobiology poses some of the most basic, profound and intriguing questions about life and human existence such as: "How did life originate and evolve?" "What is the future of life?" "Does life occur elsewhere in the Universe?"

CODA

As a scientist I have rejoiced in the opportunity to search for and occasionally discover some scientific truths that are part of the complex tapestry of nature. I have had a thrilling career. I have enjoyed my interactions with all sorts of wonderful people from professors, mentors, undergraduate, graduate and post-doctoral students, colleagues at UW and elsewhere in the global science community with whom I have enjoyed associating.

Figure 12. Wendy, Greg, Sonja and Jim Staley family

I am most grateful to my adorable wife Sonja, lovely daughter Wendy who married Mark Colbert, our enthusiastic late son Greg, our clear-headed grandson, Jack Colbert and my extended family during my long career. I am also grateful to all of those with whom I have traveled intellectually and figuratively in seeking and discovering some scientific and human truth.

In particular I thank my mentors: Robert Pengra whose undergraduate course enticed me into microbiology, William Boyd who introduced me to polar biology, Robert Hungate at UC Davis in whose lab I worked and John Sherris who recruited me to the University of Washington.

I appreciate the helpful suggestions from Eugene Nester, Ron Merrill and Wendy Colbert although I take full responsibility for the final draft. I thank Robert Staley for selected photos.

Table 1. Names of bacterial taxa described by Staley lab

Phylum	Genera and Species	Current status	Reference

Verrucomicrobia (Hedlund, Gosink, Staley 1996 IJSEM 46:960)

Prosthecobacter fusiformis (Staley, deBont, deJonge 1976 Ant van Leeu 42:333)
Prosthecobacter dejongei (Hedlund, Gosink, Staley 1997 Ant van Leeu 72:29)
Prosthecobacter debontii (")
Prosthecobacter vannervenii (")

Planctomycetes
"*Pasteuria ramosa*" (Staley 1973 Can J Micro 19:609) now Pirellula staleyi
Planctomyces maris (Bauld, Staley 1976 J Gen Micro 97:45)
"*Scalindua richardsii*" (Fuchsman et al 2012 FEMS Micro Eco 1)

Bacteroidetes
Polaribacter irgensii (Gosink, Woese, Staley 1998 IJSB 48:223).
Polaribacter franzmannii (")
Polaribacter filamentus (")
Polaribacter glomeratus (")
Gram + *Planococcus mcmeekinii* (Junge et al., 1998 Sys App Micro 21:306)
Rhodococcus zopfii (Stoecker, Herwig, Staley 1994 IJSB 44: 106)

Proteobacteria
Prosthecomicrobium pneumaticum (Staley 1968 J Bact 95:1921)
"*Prosthecomicrobium enhydrum*" now *Vasilyevaea enhydra* (Yee et al 2010 IJSEM 60:2960)
Vasilyevaea mishustinii (")
"*Prosthecomicrobium litoralum*" (Bauld, Bigford, Staley IJSB 33:613) now *Bauldia litoralis* (Yee et al 2010 IJSEM 60:2960,)
Bauldia consociatum (")
Prosthecomicrobium hirschii (Staley, IJSB 1984 34:304)
Proposed: "*Prosthecodimorpha hirschii*" submitted
Ancalomicrobium adetum (Staley 1968 J Bac 95:1921)
Aquabacter spiritensis (Irgens et al 1990 Archives Microbiol 155:137)
Octadecobacter arcticus (Gosink, Herwig 1997 Staley Sys App Bac 20:356)
Octadecobacter antarcticus (")
Colwellia demingiae (Bowman et al 1998 IJSB 48:1171)
Colwellia hornerae (")
Colwellia rossensis (")
Colwellia psychrotrophica (")
Cycloclasticus pugetii (Dyksterhouse et al 1995 IJSEM 45:116)
Neptunomonas naphthovorans (Hedlund et al 1999 AEM 65:251)
Vibrio cyclotrophicus (Hedlund, Staley 2001 IJSEM 51:61)
Polaromonas vacuolata (Irgens, Gosink, Staley 1996 IJSB:46:822)
Psychromonas ingrahamii (Auman et al 2006 IJSEM 56:1001)
Psychromonas boydii (Auman et al 2010 IJSEM 60:84)
Uncertain taxon: *Enhydrobacter aerosaccus* (Staley et al. 1987 IJSB 37:289)

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The History of the National Collection of Type Cultures (NCTC) and the Collection as a Resource for Systematists and the Wider Scientific Community

Barry Holmes

Frantisek Král initiated the first bacterial culture collection in the world, in 1890 in Prague, though he did not offer a supply service. It was the NCTC, established in 1920, that was the first collection in the world to offer a supply service of bacterial cultures, with about 2,000 cultures distributed in the first year and the first catalogue published in 1922. The NCTCs Order Book from 1920 confirms that Dr (later Sir) Alexander Fleming was one of its earliest customers. The early history of the NCTC has been published previously (St John-Brooks, 1944, 1945) and the history of the first 50 years of the NCTC was recorded in a publication celebrating that anniversary (Anon, 1971); this publication is the source of some of what follows. The author was employed in the NCTC from 1971 to 2012, so this article consolidates 92 years of NCTC history in a single record.

The Medical Research Committee (later Council, MRC) had for some time considered the setting up of a type culture collection from which authentic micro-organisms could be obtained from a trustworthy source for use in scientific work. Prior to World War I, the principal sources of supply were outside the United Kingdom, including the Institut Pasteur in Paris, the Král collection (later moved to Vienna) and the American Museum of Natural History in New York (in 1925 to become the American Type Culture Collection, ATCC). The NCTC started on the 1st January 1920 under the directorship of Dr (later Sir) John C. G. Ledingham, who was Chief Bacteriologist to the Lister Institute of Preventive Medicine in Chelsea, London (Figure 1). The Lister Institute provided the accommodation and the Medical Research Council provided the equipment and staff. The first Curator was Dr R. St John-Brooks, and he was supported by an Assistant Curator, Mabel Rhodes. The nucleus of the Collection comprised the some 200 cultures already held in the Lister Institute Private Collection; these included Escherich's original strain of *Escherichia coli*. The first accessions were Sir Frederick Andrewes' strains of *Shigella flexneri*.

Though unpublished, records of Mabel Rhodes (1945) survive and paint an interesting picture. She records that when they first occupied the accommodation at the Lister Institute it "was one of the largest and most pleasant in the building" but "In the way of equipment it contained nothing but a telephone so until the necessary equipment could be obtained the fitting out was done by scrounging and borrowing from other departments though everyone was most helpful in setting up the Collection." She records how "Paraffin wax was used almost from the first for sealing cultures but, owing to its messiness, other methods were tried, sometimes with disastrous results." She recalled how "The use of semi-solid serum agar under paraffin reduced the subculturing of the *Neisseria* from once a week to once in two months."

In these early days, there were no special facilities for the handling of hazardous pathogens such as *Shigella* species. In 1922, a requested culture of *Francisella tularensis* was received with the "advice that it should be thrown down the sink as so many workers had gone down with the disease" (Rhodes, 1945). In subsequently handling it, the Curator, Assistant Curator and another microbiologist, Dr H. Schütze, all became infected, probably from handling guinea pigs experimentally infected with the organism. Not surprisingly, experiments on animals with this organism were discontinued.

In 1930, Dr Ledingham became Director of the Lister Institute leaving Dr St John-Brooks in charge of the collection. Initially, cultures were maintained and distributed by subculture but Dorset's egg medium, sloped in bijou bottles, was soon adopted. In 1933, experiments began on the use of freeze-drying for the long-term preservation of bacterial cultures using the Homer Smith method, but the results were disappointing (Rhodes, 1945). However, in 1934, a technique was adopted as described to Dr St John-Brooks by Professor Alfredo Sordelli who was visiting from Buenos Aires at the time. Cultures were freeze-dried over phosphorous pentoxide, and, from 1934 onwards, almost every strain in the collection was dried in duplicate by this method. Cultures were still, however, distributed in sub-culture, with the freeze-dried tubes held in reserve from which to prepare subcultures.

The NCTC received many visitors over these years, the most notable being "Queen Elizabeth (*sic*) of the Belgians and Professor Einstein" (Rhodes, 1945). Apparently the press was rather troublesome at times, writing frivolous articles about the Collection (Rhodes, 1945).

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Early on in the NCTC, it became apparent there was a significant demand in the United Kingdom (UK) for the supply of cultures of microfungi. The NCTC therefore collaborated with the British Mycological Society for their assistance in the maintenance of microfungi, many strains of which were then held in the NCTC collection. Under a centralisation policy at the Lister Institute the NCTC collection, similar to that at ATCC, came to include microorganisms of medical, veterinary, agricultural and economic interest including diverse organisms as both pathogenic and saprophytic fungi, phytopathogenic bacteria and yeasts.

By the end of its first year of operation, the NCTC held about 800 cultures. Five years later the number held had increased to 1,500, after 10 and 15 years to 2,500 and 3,500, respectively, and by 1939 it numbered some 4,300 strains. Subsequent to publication of the first catalogue in 1922, succeeding editions were published in 1925, 1931 and 1936. In 1938, just prior to the advent of World War II, 6,397 cultures were distributed, of which 45% were for scientific research, 20% went to colleges and schools for teaching purposes and 35% to workers engaged in technology. Of those cultures, 46 were dispatched abroad (Africa 6, Asia 11, Australasia 2, Europe 23, North America 2, South America 2).

In 1939, the NCTC was transferred from Chelsea to the Lister Institute farm laboratories at Elstree, Hertfordshire (site subsequently occupied by Bio Products Laboratory, Dagger Lane), where the space available was limited (Figure 2). The NCTC faced a difficult period, and, by 1944, held about 5,000 cultures, imposing a considerable strain on the staff and space available to maintain the cultures. The war conditions imposed other difficulties. Although formal export controls in the UK were not introduced until 1993 following the first Gulf war, during World War II the Postal and Telegraph Censorship Department in 1944 intercepted a culture of *Penicillium notatum* NCTC 4222 which had been dispatched to the Sociedade Industrial Farmaceutica in Lisbon. The culture was returned to the NCTC and Dr St. John-Brooks wrote to the censor advising that he was not aware of any embargo on the sending of cultures of Sir Alexander Fleming's strain of *P. notatum* out of this country. However, the Allies did not want the enemy to develop penicillin, for if they had done so then this may have had a profound effect on the course of the war. The interesting story of penicillin in Europe during World War II is recounted by Sharma (2009). There is a similar report of a culture being intercepted having been despatched to "the Argentine" to an institute whose director was on a government blacklist that the NCTC staff had no prior knowledge of.

Come 1945, the war ended, and the following year Dr St. John-Brooks retired and Dr Samuel Tertius Cowan took his place as Curator from 1st January 1947. At this time, the MRC took the view that the NCTC should confine itself to bacteria of medical and veterinary interest. Characterisation of all the cultures in the collection was begun, and, during the period 1946-1950, groups of microorganisms not meeting the NCTCs new remit were transferred to relevant institutes where they formed the nuclei of several of the UKs other national culture collections. In some cases, the original NCTC number was maintained, thus the National Collections of Industrial and Marine Bacteria (NCIMB) which were to form then in Teddington (now in Aberdeen) hold the same strain of *Staphylococcus aureus*: NCTC 6571 ≡ NCIMB 6571. The NCTCs culture holding was reduced to about 3,000. Following a recommendation in 1947, the United Kingdom National Committee of the British Commonwealth Collections of Microorganisms (UKNC) was established to coordinate the activities of the then existing collections of microorganisms.

Events leading to the creation of the Emergency Public Health Laboratory Service in 1939 have been described by Wilson (1948). The Public Health Laboratory Service (PHLS) itself came into being in 1946 with its central laboratory in Colindale at what had previously been the Government Lymph Establishment (built to facilitate the production of smallpox vaccine). It is worth noting that the Government Lymph Establishment also began its work at the British Institute of Preventive Medicine at Chelsea (later known as the Lister Institute of Preventive Medicine) in 1898, but moved in 1907 to the newly constructed Government Lymph Establishment at Hendon (Fremlin, 1946). In July 1949, the NCTC moved from Elstree to the Central Public Health Laboratory (CPHL) of the PHLS in Colindale, London. At this time some 6,500 cultures were being supplied annually. The NCTC took up residence in a "temporary" building known affectionately as "the hut". Here, in 1949 and with new equipment, large-scale freeze-drying commenced, one of the first national collections to do so, and a new policy was implemented for every strain in the collection: to check its purity, to test and record its characters (colony morphology and biochemical test results were recorded on pre-printed cards measuring 13x6 inches), to freeze-dry adequate stocks for distribution and to test at regular intervals the viability of the dried cultures and confirm their purity (a card was used for each strain to record details of successive batches and on another card the successive counts on an individual batch). This mammoth task took 10 years to complete. The millionth ampoule was produced in 1970 and to this day the NCTC cultures are largely distributed as viable freeze-dried (lyophilised) cultures in glass ampoules sealed under vacuum.

In 1960, the administration and finance of the NCTC passed from the MRC to the PHLS Board. Dr Kenneth John Steel joined the NCTC in 1959 and was soon running NCTC. This was for various reasons, not the least of which was the appointment of Dr Cowan as Administrative Director of CPHL in 1961. Subsequently, Dr Cowan also became Deputy Director of the PHLS, but then resigned as Curator of NCTC, to be replaced in 1965 by Dr Stephen Paget Lapage.

Bacterial Identification Service

Given the wide range of bacteria held and maintained in the NCTC, as in other collections, staff became well acquainted with their characteristics. Reference laboratories existed for organisms such as *Salmonella* and *Shigella* species submitted by sending laboratories to Colindale, but the NCTC provided a service for the identification of bacteria for which no reference laboratory existed. Such organisms could be atypical strains of commonly encountered species, rare species, or even new taxa. To aid medical laboratories worldwide in the practical identification of medical bacteria Drs S. T. Cowan and K. J. Steel drew on the NCTCs expertise and compiled a highly-acclaimed manual (Cowan and Steel 1965; with a second edition in 1974 and a third edition in 1993). With the formation of the NCTC Computer Laboratory (*vide infra*), the identification service operated in two sections with the Gram-positive and fastidious Gram-negative organisms continuing to be identified by a traditional approach and without the benefit of computer assistance. This national referral service was first offered in 1965 and was provided by Mr. Jerry J. S. Snell using the biochemical test result tables in Cowan and Steel's Manual as well as culture collection strain data. From the test results of isolates submitted for identification, Jerry improved the test result tables and produced other tables for some of the more fastidious bacteria. These appeared in later editions of Cowan and Steel's Manual. The two new species *Kingella denitrificans* and *K. indologenes* were published from this work (Snell and Lapage, 1976). Rita Legros took over managing the service in 1975, and was involved in publishing a proposal of a new species *Haemophilus equigenitalis* (Taylor et al. 1978). During this period the service started to use a computer matrix for the identification of *Corynebacterium* and closely related genera developed by Dr. I. S. Bowie and Dr. W. R. Willcox. From mid-1979 this identification service was headed by Dr Henry Malnick; he published several articles, particularly on *Anaerobiospirillum* (e.g. Malnick et al. 1989). In-house rapid enzyme/biochemical tests were introduced for fastidious bacteria that could not be grown in standard test media so that an identification could be obtained in 4 – 18 hours. The gas chromatograph (*vide infra*) for the detection of end products of glucose fermentation proved a useful additional tool for identifying *Lactobacillus* spp., anaerobic *Actinomycetes* spp. and *Propionibacterium* spp. The service was transferred elsewhere in Colindale in 1994 (*vide infra*). By this time, there had been over 20 articles published by staff and many more published by referring laboratories who acknowledged named staff or the NCTC for the identification.

Computer Laboratory

The NCTC was awarded a research grant by the Department of Health and Social Security (DHSS) in 1965 to develop a computer-based identification system, the first such grant to be awarded to the PHLS. At this time no-one could foresee how much computers would develop over the next 50 years. This project is thought to be the first application of a computer in the PHLS, for identification of bacteria applying Bayesian probabilities to the results of a series of conventional biochemical tests. The group of organisms chosen as a model was Gram-negative aerobes able to grow on ordinary nutrient agar. The Atlas Computer used was a joint development between the University of Manchester, Ferranti, and Plessey. The first machine, officially commissioned in 1962, was one of the world's first supercomputers, considered to be the most powerful computer in the world at that time, equivalent to four IBM 7094s. It was said that whenever Atlas went offline half of the United Kingdom's computer capacity was lost. Two further machines were built. The results of the biochemical tests were punched onto 8-column paper tape which was read by a Flexowriter which converted the data to an electrical signal to be sent via a telephone link to London University Computer Services (LUCS) Atlas computer. Following the computations, printouts of the results were received by post. Subsequently the PHLS invested in a series of in-house ITL minicomputers onto which the identification programs were transferred. Data were input manually via terminals to the computer, subsequently by pre-printed Optical Mark Recognition (OMR) forms, and results were printed out in-house. Over successive years probability matrices were published for Gram-negative fermentative organisms (Holmes et al. 1986a), non-fermentative Gram-negative organisms (Holmes et al. 1986b) and Campylobacters (On et al. 1996). As bacteria cannot be identified successfully unless they have been classified well, the Computer Laboratory became heavily involved in systematics, recognising and describing new taxa, often in collaboration with similar centres overseas (e.g., *Ochrobactrum anthropi*; Holmes et al. 1988). In later years, in collaboration with Dr M Costas, the use of SDS-PAGE of whole cell proteins and their computerised analysis was investigated as a means for not only identifying an organism to the species level, but assigning it to a particular electrophoretype at the same time (Costas et al. 1993a).

The rise in interest in *Clostridium difficile*, due to some significant outbreaks, also lead to the application of electrophoretyping in the investigation of outbreaks in Canada (Costas et al. 1994a) and the UK (Costas et al. 1994b). The Computer Laboratory continued to provide a probabilistic identification service until 1994. In 1993, the last full year of operation, for all the NCTC identification services combined, 1,015 cultures were received of which 926 proved pure and viable; they fell into the following categories (laboratories assuming responsibility for these organisms post-1994 given in parentheses): 1. Gram-negative, non-fermentative, non-fastidious bacteria: 419 (45%; Laboratory of Health Care Associated Infections [LHCAI]); 2. Gram-positive, fastidious and non-fastidious bacteria: 261 (28%; Respiratory and Systemic Infections Laboratory [RSIL]); 3. Gram-negative, fermentative, non-fastidious bacteria: 164 (18%; Laboratory of Enteric Pathogens [LEP]; 4. Gram-negative, fastidious bacteria: 82 (9%; retained briefly in the NCTC). Some 160 laboratories made use of the identification services in 1993.

Origins of UKNEQAS

As a condition of using the probabilistic identification service offered by the Computer Laboratory, from 1966 onwards, sending laboratories had to complete an application form which included the biochemical test results they had obtained for the unknown organism. These results, together with further results determined in the Computer Laboratory, were processed through the computer to hopefully achieve a satisfactory identification level. The identification might be achieved on the sender's results alone, a combination of Computer Laboratory and sending laboratory results or purely on Computer Laboratory results. The process frequently repeated tests carried out by the sender and differences were frequently found, often proving to be the reason the sender couldn't identify the organism in their own laboratory. As examples, there were differences between sender and Computer Laboratory in 17% of cases of acid from sorbitol and 16% for nitrate reduction. The mean was 6.1% of the tests showing differences. Only for 19% of strains tested were no differences found and in 12% of strains there were as many as three test differences. The authors (Lapage et al., 1970) alluded to the desirability of standardised methods of testing. These observations were followed up by a questionnaire to the sending laboratories, the results from which revealed laboratories were using a wide range of methods for nominally the same test. To determine the effects of these varying methods on laboratory outcomes, freeze-dried disc cultures for quality assessment of water testing for presumptive coliforms were developed in the NCTC during 1971-72. The discs were prepared from Whatman's glass fibre paper as these were easily broken up. Freeze-dried disc cultures for quality control surveys were developed during 1972-74. Whatman's antibiotic assay discs were inoculated with a bacterial suspension and then freeze-dried. The discs were then sealed, in air, between layers of various materials of which heat-sealed, plastic-coated aluminium foil ultimately proved best for short-term preservation. Accordingly, a pilot external quality assessment (EQA) distribution was made comprising four cultures on discs sealed in foil. At that time the cultures could be put in the ordinary post in a plain brown envelope, with a survey sheet on which the recipient laboratory could report their findings. Following this initial trial, Dr SP Lapage discussed his concerns and results with PHLS senior management. Dr P B Crone from the Sunderland Public Health Laboratory then based himself initially in the NCTC analysing the results of further distributions. Wider collaboration in an initial *ad hoc* manner took place between various interested parties, before a Steering committee was formed with the collaboration of Joan Stokes at University College Hospital (UCH), the PHLS, the Department of Health (DoH) and the Societies and Colleges. The Quality Control Laboratory (QCL) was subsequently established in Neasden Hospital with Dr Peter Crone as its first Director. When Dr Crone retired, QCL was merged with the Standards Laboratory for Serological Reagents as the Division of Microbiological Reagents and Quality Control (DMRQC) with Dr Philip Gardner as Director, and moved to a building on the Colindale Hospital site. The QCL function was integrated across the Division. After Dr Gardner retired, Dr Tony Taylor became Director. The QCL functions were taken over by Mr Jerry JS Snell (a former NCTC medical laboratory scientific officer) as Scheme Organiser in 1985 and the laboratory moved into the new CPHL at this time. At the behest of DoH, QCL was subsequently split off as QAL (Quality Assurance Laboratory) with Jerry Snell made Director in 1991 (the rest of DMRQC briefly became DMR and then LMR [Laboratory of Microbiological Reagents] until closure in 1994). By 2010, the UK National External Quality Assessment Scheme (UKNEQAS) had created some 22 new microbiology schemes in 10 years and some 52 countries were participating in one or more of these schemes.

Molecular biology

Toward the end of 1966, Robert J. Owen was appointed under a three-year MRC grant to study bacterial DNA and officially became a member of the NCTC staff early in 1970. The work initiated under the MRC project continued with investigation into improved methods of DNA extraction and ways of calculating G+C content (Owen and Hill 1979).

Base composition determinations were performed for in-house research projects and as a service provided to meet any outside requests, including training. Also DNA-DNA hybridisation by various methods and genome size determinations were on-going activities as part of the NCTC systematics research - notably on Alteromonads, Flavobacteria, Pseudomonads, then increasingly on Campylobacters. The NCTC was fully equipped for radioisotopic labelling work with a dedicated laboratory. Joint projects included one with the PHLS Mycology Reference Laboratory, then at the London School of Hygiene and Tropical Medicine, which investigated the DNA contents of dermatophytes using the NCTC analytical ultracentrifuge. In the 1980s, the DNA work developed extensively with the availability of new procedures particularly for strain discrimination (molecular typing) e.g. restriction endonuclease analysis, Southern blot hybridisation, pulsed field gel electrophoresis, PCR Restriction Fragment Length Polymorphisms (RFLP) analysis and later on partial DNA sequencing (Owen 1983, 1989). This was important in identification as the concept of the species was refined and population genetics became a basic principle. Work on *Campylobacter* that included setting up phage- and sero-typing schemes, as well as molecular typing, had significant funding from the DoH and Food Standards Agency (FSA). On-going opportunities for visitors to learn the techniques used for all aspects of the work were available every year in the form of 12-month placements for students from UK universities to complete a project for their degree and for qualified graduates from EU regions to do a period of advanced study funded by the European Commission (mainly from Spain). Much developmental work was also carried out on rapid detection and identification by means of PCR assays for *Campylobacter* and *Helicobacter* species. Another notable scientist laying the groundwork in these developments was Dr. David Pitcher. He described DNA extraction using Chelex, and Guanidine thiocyanate (Pitcher et al. 1989) as well as DNA sequencing using the LKB Macrophor (Pharmacia Biotechnology Inc.). Dave Pitcher applied himself particularly to ribotyping, RFLP analysis and to Southern blotting and hybridisation using non-radioisotopic (biotinylated) probes. He used numerical analysis with the application of UPGMA and the Dice coefficient (Soto et al. 1991). In 1994 much of this work ceased and Robert Owen, Deputy Curator of NCTC at the time, was transferred to LEP to head a *Helicobacter* reference unit.

Gas-liquid chromatography Laboratory

In the early 1970s, there was promise in differentiating bacteria by analysis of their metabolites, cellular fatty acids and other complex cellular macromolecules using gas-liquid chromatography (GLC). This was because of the limits of identification of bacteria using the then 'standard' biochemical tests even where these were linked with sophisticated numerical analysis of the results. The demand for GLC analysis arose due to the occurrence of lesser known Gram-positive and Gram-negative bacteria in infections of immune-compromised patients. Accordingly, a Medical Research Council (MRC) grant was successfully acquired with which to purchase a GLC and to support a research scientist to develop the methodology. The instrument obtained was a Pye Unicam GCV (Gas Chromatograph Versatile) equipped with a flame ionisation detector and a DP 101 computing integrator recorder for data analysis. Traces were recorded on a Philips PM 8100 flat-bed recorder. The first research scientist appointed was Dr John E Hine who came from Brisbane, Australia, to take up the position from August 1975 to August 1978. The work done during this period focused on metabolic by-products. Some useful results were yielded with Gram-positive coryneform bacteria but less useful results were obtained with pseudomonad-like bacteria, possibly as they are more oxidative and convert sugars to carbon dioxide and water, rather than short chain fatty acids. In retrospect, work on cellular fatty acids and other complex cellular macromolecules should have begun earlier. However, the GLC research in the NCTC was undertaken in a new area where the science and the scientific techniques were not fully familiar to those involved, all of whom were microbiologists and not chemists. The NCTC laboratories at this time were also not equipped for complex chemistry and work on cellular lipids would have required the use of flammable and possibly toxic solvents. Leaving the project with a sole researcher was also unhelpful; it could have been more fruitful had the work been carried out in collaboration with a laboratory that had more experience in such chemical analysis and the equipment required for such work, thereby facilitating a team approach. John Hine was followed by Mrs Jane H Barnard, who had joined the NCTC in March 1977 and would head the GLC Laboratory until September 1980. In January 1979 Mrs Susan M Leaper joined the NCTC until 1982. She developed an interest in using the NCTC GLC for the differentiation of *Campylobacter* species (Leaper and Owen 1981). Although further developmental studies with the instrument were not pursued, the instrument proved a useful additional tool for identifying certain organisms, by detection of the end products of glucose fermentation, in the bacterial identification service (*vide supra*). The machine fell into disuse once the NCTC started referring all the anaerobes to the Anaerobe Reference Laboratory then at Luton, for post freeze-drying authentication checks. Eventually the instrument was disposed of and the laboratory (on the ground floor of the Tower building) closed.

Molecular Identification Research Unit

The NCTC Molecular Identification Research Unit was established in 1983 under the direction of Dr. Menelaos Costas. The major research interest was centered on the development of molecular methods (both protein and nucleic acids) for the identification and typing of pathogenic bacteria. The techniques used included high-resolution 1- and 2-D polyacrylamide gel electrophoresis for total bacterial protein separation and agarose gel electrophoresis for separation of nucleic acids. The resulting bacterial "fingerprints" were then analysed using an LKB Laser Scanner (Densitometer) linked to a sophisticated computerised numerical analysis package (developed in-house). The equipment was of the most modern and sophisticated then available and included two laser densitometers and powerful microcomputers. The Unit proved an international centre of excellence and attracted major grants from both the EEC and the USA. A number of scientists (both national and international) interested in furthering their technical competence and understanding of these techniques were trained at the Unit. The analytical methods employed were at the leading edge of technology. The Unit provided electrophoretic and analytical technology which was not available elsewhere. It was one of only a few centres specialising in the computer analysis of complex electrophoretic patterns (both protein and DNA) of pathogenic bacteria. A four-year grant for protein research, partly provided through the EEC, enabled the Unit to make rapid progress especially in software for analysis of electrophoretic protein patterns. Collaborations were undertaken with other groups, primarily based in Belgium, in both 1- and 2-D protein research via this grant. Three further major grants were also awarded to the Unit, from the United States (from Norwich Eaton Pharmaceuticals and Proctor & Gamble [2 awards]). These grants were originally for electrophoretic protein analysis of *Helicobacter pylori*, but were then extended to both protein and nucleic acid analyses of this pathogen. Much of the work was undertaken in collaboration with Dr. R.J. Owen, who had a major interest in *Campylobacter* and *Helicobacter* species. The results of the research efforts of the Unit have provided the material for numerous research papers published in international journals (e.g. Costas et al. 1993b). The Unit closed in 1994.

Plasmid Laboratory

The NCTC Plasmid Collection was established in 1985 under the direction of Dr. Victoria Hughes. The nucleus of the collection was the considerable collection of medically important plasmids and supporting strains amassed by Professor Naomi Datta FRS and colleagues at Hammersmith Hospital between 1960 and 1984. The Datta collection, some 10,000 cultures, had been maintained in nutrient agar stabs at ambient temperature and comprised clinical isolates, mostly *Enterobacteriaceae*, but also pseudomonads and plasmids (mostly R plasmids) derived from those strains, held in *Escherichia coli* K12 of various genetic backgrounds. In addition to Incompatibility (Inc) group reference plasmids, a wide range of plasmids of various Inc groups and plasmid variants (generated by *in vivo* recombination, gene insertion or deletion) facilitated the classification of the multiple resistance plasmids commonly isolated. Also included in the NCTC Collection are plasmids from many other laboratories, plasmid-free genetically marked strains, plasmid DNA molecular weight standards, strains with transposons in plasmids or the host chromosome, and plasmids whose resistance genes have been characterised. Preservation, quality control and supply protocols were established for accessions and a database developed to generate a catalogue of the strains and their uses. The cultures were accessioned into the NCTC by their own set of reference numbers and were listed separately in the 1994 edition of the NCTC Catalogue of Strains. Later characterisations included the Murray Collection of pre-antibiotic era bacteria which facilitated studies of the molecular evolution of resistance plasmids and their genes and the accession of Gram-positive genera and their resistance determinants when these infections gradually became more prevalent in both clinical and community settings. The laboratory became the medical branch of the CEC-funded European Resource Centre for plasmid-bearing bacterial strains in collaboration with the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), which supplied plasmids of biotechnological relevance. Later the plasmid laboratory provided the location for, and contributed to, a BBC/Open University TV broadcast on microbial genetics and antibiotic resistance.

Dr. Victoria Hughes left in 1988. She was succeeded in 1990 by Dr John Stanley. With the agreement of the then Director of the CPHL, Dr Morag Timbury, the unit was renamed as the Molecular Genetics Unit within the NCTC, to focus on plasmids as molecular composites of transposable elements and extrachromosomal replicons, as well as on emerging molecular genetic approaches to the epidemiological typing of a variety of significant bacterial pathogens. Within the NCTC, Dr. Stanley pursued DNA-based studies in cooperation with the unit of Dr. Robert Owen, concentrating on speciation and typing of members of the genera *Campylobacter* and *Helicobacter*. The techniques employed included high resolution genotyping, plasmid profiling, 16S rRNA gene typing, use of various species-specific DNA probes and other studies of genetic diversity. Three new species were described: *Campylobacter helveticus*, *Helicobacter canis* and *H. pullorum*. Studies of the genus *Salmonella* were carried

out in cooperation with the unit of Dr. John Threlfall in the Laboratory of Enteric Pathogens (LEP) at the CPHL and the Swiss National Reference Laboratory for Foodborne Disease in Berne. Three members of the technical staff of the unit (Meeta Desai, Namoos Baquar and Dennis Linton) were awarded PhDs in recognition of their published contributions to *Campylobacter*, *Helicobacter* and *Salmonella* research. In 1994, Dr. Stanley and the above staff members were relocated to the Virus Reference Division under Dr. Philip Mortimer, to form half of a new Molecular Biology Unit whose remit extended to CPHL as a whole. The NCTC was then left with maintenance of the plasmid collection, despite having no resources or special expertise with which to do so. The collection was maintained through the good offices of LEP at Colindale with advice on appropriate growth media and conditions to conserve the plasmids and then checking of new ampoule batches that the host strains contained plasmid DNA of the expected molecular weight(s). Cultures from the collection remained available to order from the NCTC.

Mycoplasma Reference Laboratory and NCTC

At its inception in 1966, the PHLS Mycoplasma Reference Laboratory (MRL; Director Dr. Basil Andrews) was housed within the Virus Reference Laboratory at Colindale and then, from 1976, at the Norwich Public Health Laboratory. The Reference Laboratory had a continuing close collaboration with the NCTC. Its collection of reference *Mycoplasma* cultures were lyophilised by, and supplied from, the NCTC. These cultures had their own set of reference numbers and were listed separately in the NCTC Catalogue of Strains. Culture suspensions would be prepared in the MRL and transferred to the NCTC to be freeze-dried. Ampoules of new batches would be sent back to MRL for checking that the correct organism had been freeze-dried and that the viability count and culture-purity were satisfactory. This arrangement was relatively simple during the period 1966-76, but became more difficult when MRL relocated to Norwich and culture suspensions had to be driven to the NCTC at Colindale. It was always intended that when the new CPHL was built the PHLS Mycoplasma Reference Laboratory would move there from Norwich. A suite of rooms had been dedicated for the Laboratory. However, such were the changes in needs from inception to completion of the new building that the suite of rooms was re-allocated to more pressing work. The reference function did indeed move to the new building in 1984, but as an NCTC unit, under Dr. Ronald Hubert Leach and renamed the Mycoplasma Reference Facility. It subsequently became in 1990 a designated FAO/WHO Collaborating Centre for Reference and Research on Mycoplasmas. Dr. Leach retired officially in July 1991 but was then reappointed for three successive years as an honorary microbiologist and retired fully in July 1994. Dr. David Pitcher gradually took on leadership of the Facility over those years. Like other areas of the NCTC, the Facility undertook studies of systematics and described new taxa (e.g. *Mycoplasma auris*, *M. cottewii* and *M. yeatsii*; DaMassa et al. 1994). In October 1994 the Facility, then headed by Dr. Pitcher, was relocated within the then Respiratory and Systemic Infections Laboratory (RSIL) at Colindale. Initially, it had been intended that the FAO/WHO Collaborating Centre functions were to be shared between RSIL (for curatorial functions) and the NCTC (for culture ordering and supply functions). However, it had become accepted that only a few mycoplasmas were of significance to human health so the Facility staff were gradually reassigned within RSIL to other specialities, deemed more pressing than mycoplasmas. Dr. Pitcher continued to provide some support, performing certain tests such as growth inhibition and G+C content calculation for new *Mycoplasma* species that were submitted to the NCTC for accession. However, the NCTC was left with maintenance of the *Mycoplasma* collection, despite being left no resources or special expertise with which to do so. The collection was maintained through the good offices of Mycoplasma Experience Ltd., who would prepare the suspensions and send them to the NCTC by courier for freeze-drying, whilst a Liverpool University department performed the quality control checks on new ampoule batches.

Other NCTC activities

The NCTC scientific and technical staff were also fully involved in all the culture collection routine activities such as dealing with world-wide queries and orders for cultures, accessioning, stock control and the re-drying programme, viability checks and weekly quality control meetings (the so-called 'plate party' was held every Thursday afternoon where all senior staff would examine all culture plates). Research and development was also carried out to improve preservation. Following trials carried out by Keith Redway, inositol replaced glucose for NCTC freeze-drying *circa* 1973, using either inositol broth or inositol serum. Serum was used routinely for most organisms, and broth for enterobacteria to avoid possible immunological change or damage. The trials showed a general improvement in long-term survival using inositol for many cultures. Attempts were also made to improve the viability of problematic anaerobes and L-drying (Liquid drying, i.e. with no freezing of the culture) was developed for routine preservation of *Helicobacter* species. The L-drying method used in the NCTC was based on methods developed by Annear and others, dating back to 1958.

NCTC national activities and relations with other UK collections

A little over 10 national microbial collections existed in the 1970s. Two subsequently closed: the National Collection of Wood-Rotting Fungi (NCWRF) and the National Collection of Food Bacteria (NCFB; incorporating the National Collection of Dairy Organisms, NCDO). Their collections were re-homed respectively by CABI (Centre for Agriculture and Biosciences International) and the National Collections of Industrial and Marine Bacteria (NCIMB; at one time separate collections). The remaining eight collections are detailed in Table 1 with their full titles (NCPV did not come into being until 1999). As the various UK culture collections largely had different funding sources, collaboration between them was on an informal basis. For many years their joint forum was the UKFCC (United Kingdom Federation for Culture Collections) with occasional meetings and a regular newsletter (Issue 29 was published in 1998). The NCTC and NCPF, however, were both part of the PHLS so there was particularly close collaboration between them. All NCPF cultures that could be preserved by freeze-drying were lyophilised by the NCTC. Some of the more popular NCPF cultures were also held in stock by the NCTC to provide more of a 'one-stop shop' to several customers.

The need to further develop the collections was recognised by advisors to the UK Government (Whittenbury, 1994) resulting in the funding and establishment of the UK National Culture Collection (UKNCC), bringing together expertise and co-ordinating some essential activities (effectively replacing UKFCC, which ceased to be). A critical mass was created to enable the member collections to achieve much more together. An on-line catalogue was created of the consolidated holdings of all the collections, manuals and hard copy catalogues were printed as well as promotional literature, and trade stands were manned in commercial exhibitions at international conferences; UKNCC even had its own logo adopted by all the constituent collections. Unfortunately, the UKNCC funding was limited and once used up the collections started to lose their interdependency.

The PHLS was subsumed within the Health Protection Agency (HPA) in 2003. Of the nine national collections in the UK at this time, four now belonged to the same organisation: ECACC and the NCPV, both at HPA Porton, the NCPF at Bristol and the NCTC at Colindale. Although they initially collaborated informally, all subsequently became consolidated as the Health Protection Agency Culture Collections (HPACC), with a single director and website etc. (but *vide infra*). ECACC was by far the largest of the collections and as the collections became more integrated, the ECACC way of doing things prevailed. The NCTC ceased processing orders, responding to technical inquiries and having direct contact with customers as these activities became centralised at HPA Porton during 2010. By August 2010, all orders were being processed at Porton, but still being despatched from Colindale. The NCTC at Colindale became a 'production platform'. It was planned that the NCTC would leave Colindale altogether (away from the Colindale reference laboratories where lie expertise on the clinically important organisms of the NCTC collection) and relocate to HPA Porton in 2012. The NCTC space at Colindale was given up and plans made for its re-use once the NCTC had vacated it. It subsequently transpired that none of the NCTC staff would relocate to Porton, but it was by then too late for them to remain at Colindale as alternative plans had been made for the space. In the end, the NCTC was found a home at the National Institute of Biological Standards and Control (NIBSC; also part of the HPA at this time) not too far away at South Mimms. Transfer of production and the drying record cards to NIBSC took place in April 2011. Being only one year away from retirement, the Head of NCTC remained as the sole member of the NCTC staff at Colindale. The 1950s secondary drier, now replaced by two new custom-built secondary driers (Figure 3), was abandoned at Colindale at this time (see Figure 4; the longevity of this machine resulted from the fact that even into the new Colindale building from 1984, the NCTC continued to have its own workshop, including a lathe, so that all machines were regularly serviced by the NCTC staff and replacement parts could be manufactured as required). Following the move to NIBSC, the ampoule stock was still at Colindale so the NCTC laboratory staff from NIBSC had to continue coming to Colindale to pack and despatch them to fulfil customer orders. They also brought newly-produced ampoules (after passing quality control checks) from NIBSC to add to stock. The Head of NCTC was on hand to supervise packing of eventually all ampoules in preparation for their transfer to the warehouse at Porton, which was still planned to go ahead. Following commissioning of a new purpose-built warehouse at Porton, the ampoule stocks were transferred from HPA Colindale to HPA Porton in September 2011; after this Porton took over packing and despatch of ampoules.

NCTC international activities

Senior NCTC staff long participated in framing the rules that governed bacterial nomenclature. R. St. John-Brooks belonged to the Commission on Nomenclature and Taxonomy; he was one of two Permanent Secretaries of the International Committee on Bacteriological Nomenclature and a co-author in 1948 of the Bacteriological Code, now the International Code of Nomenclature of Bacteria.

Sam Cowan was Secretary of the Editorial Board producing the 1958 edition, whilst Stephen Lapage was principal author of both the 1975 and 1990 revisions of the Code.

Dr. Stephen Lapage, together with other noteworthies of the time, like Peter Sneath at the University of Leicester, engaged widely with international initiatives in bacterial systematics. A consequence of the development of stable microbial classifications was a requirement to rationalise bacterial nomenclature. Both Lapage and Sneath played significant roles in the work of the International Committee on Systematic Bacteriology that resulted in a most important innovation, a new starting date for bacterial nomenclature (1st January 1980; previously it had been 1st May 1753). Names of bacteria with poor descriptions and no recognisable type strain would lose standing in nomenclature. A committee was appointed to oversee the task of compiling the Approved Lists of Bacterial Names, retaining only those taxa which were adequately described and with type strains. This committee initially comprised just three people, one of whom was S. P. Lapage. The committee produced an edited first draft in 1976 and the Approved Lists were published and came into effect in 1980 (Skerman et al. 1980). If one takes, as an example, the genus *Pseudomonas*, in Bergey's Manual of Determinative Bacteriology 8th edition (1974) 265 species are mentioned; only 29 species are fully described (only about 10% of named species, those extensively characterised), 74 species are incompletely described but with extant type strains, 112 species are incompletely described and without extant type strains, there are 20 species that might be more appropriately included in other genera and 30 species in other genera that might be more appropriately included in *Pseudomonas*. In 1980, just 87 species were retained in the genus, only those extensively characterised and with extant type strains.

Lapage (and Sneath) were also heavily involved with the Bergey Trust, the organisation responsible for Bergey's Manual of Systematic Bacteriology, the definitive handbook of descriptions of all bacterial species at the time of publication. Several NCTC staff contributed to various subcommittees of the now International Committee on Systematics of Prokaryotes (Table 2). Cowan (1978) compiled a dictionary of microbial taxonomy, edited by Roland Hill, which has been widely used by microbial systematists and others throughout the world. NCTC staff members have also contributed widely to bacterial systematics (the author was the recipient of the 1999 Bergey Award) through numerous scientific publications, 129 in the first 50 years to 1970 and a grand total of 604 by 2012. The NCTC was extremely successful, in relation to its size, in obtaining significant grants and other funding input during its peak years from 1965 to 1995, mainly from the MRC, EU, DoH and FSA. It was this funding that was the basis for the high publication rate over those years, not only on research but also book chapters on methods. Many publications were written with overseas collaborators, in particular with the Centers for Disease Control in Atlanta and Laboratory of Microbiology of the University of Gent (LMG). There was also excellent collaboration between other culture collections, particularly the Culture Collection, University of Göteborg, Sweden (CCUG), the DSMZ and Japan Collection of Microorganisms (JCM). In the case of JCM, this collaboration extended to the Head of NCTC in 2010 climbing to the summit of Mount Fuji with two staff members from JCM (Figure 5). The NCTC is also a member of the European Culture Collections' Organisation (ECCO) and the World Federation for Culture Collections (WFCC; the NCTC being a member since WFCC's inception in 1970). The latter pioneered the development of an international database on culture resources worldwide, the result of which is the WFCC World Data Center for Microorganisms (WDCM), which also hosts the WDCM Reference Strain catalogue. The NCTC staff also joined the Task Force on Biological Resource Centres established by the OECD's Working Party on Biotechnology, which in 2007 resulted in the publication of the OECD Best Practice Guidelines for Biological Resource Centres.

October 1994 and a change in fortunes

During the early 1990s, LMR had become increasingly under scrutiny as many of the reagents it produced were also produced commercially. Consideration was being given as to whether LMR should therefore continue with its remit. At this time also, the NCTC had reached a peak, from 16 staff in 1970 it had risen to 38 (including students: Molecular Biology/Molecular Identification Research Unit [13], Identification Services [6], General Services [collection maintenance, 4.5], Mycoplasma Reference Facility [4.5], Plasmid Laboratory [5] plus Curator, Deputy Curator and 3 Office staff; Figure 6). For the financial year beginning in 1994, cuts in government spending in support of the Health Protection Agency (HPA) were announced. Accordingly, economies had to be identified and it was decided that the majority of the cuts would fall on LMR (to close altogether) and the NCTC. It will already have been noted above that several of the NCTC Units either closed or were transferred in 1994; posts were redundant and staff at risk. Some staff members lost their jobs, but most were found alternative positions and continued their scientific careers. The NCTC had been proud that not only did it have several of its staff with PhDs, but it also boasted three with DScs (Roland Hill, Barry Holmes and Ronald Leach). Now it found itself reduced to only eight members of staff (Figure 7).

Menelaos Costas was appointed the new Curator of NCTC, but then immediately seconded to head the Media Production Department (where he would remain, ending his scientific career). The NCTC now had effectively seven staff and it began a new commercial era, maintaining the collection and promoting culture sales. All research in the NCTC came to an end at this time (but *vide infra*), although some long outstanding systematics projects begun in collaboration with mostly overseas colleagues were eventually written up and published (e.g. Foster et al. 2004; Holmes et al. 2006, 2013, Vandamme et al. 2006). In 1999, Dr. Costas became fully established in the Media Production Department and Barry Holmes became Head of NCTC. The past NCTC Curators are illustrated in Figure 8.

Decline in microbial taxonomy in the UK

The NCTC was not the only centre of systematic excellence to suffer. The MRC Microbial Systematics Unit at Leicester University, headed by such as Professor P.H.A. Sneath and Dr. Dorothy Jones, was dissolved in 1975. Subsequently, the Unit effectively became the Microbiology Department in the newly set up Leicester Medical School where some systematics work was continued (e.g. Barrett et al. 1994). Another centre of systematics excellence was the Department of Microbiology, AFRC Institute of Food Research, Reading Laboratory under Dr. Matthew David (Dave) Collins (home also of the National Collection of Food Bacteria, NCFB). They also described new taxa (e.g. Collins et al. 2004), but this laboratory too was to close in 2004. In evidence submitted by the Society for General Microbiology in 2008 to the House of Lords Science and Technology Committee's Follow-up Inquiry on Systematic Biology Research and Taxonomy, it is reported that in the years 2000 - 2002, some 8 - 9% of papers in the International Journal of Systematic and Evolutionary Microbiology (IJSEM) had corresponding authors from the UK. By 2007 this had fallen to 2%. Examination of the January 2008 issue of IJSEM, for example, showed that only two papers describing new species came from UK laboratories.

An electronic catalogue of culture data

The last hard copy catalogue had been published in 1936. By the late 1960s a new edition was planned with much of the revision to be undertaken by Dr. R.J. Owen. There was no alphabetical listing of the NCTC cultures before 1970. There were numerical lists but these were out of date and included numbers of cultures such as fungi, plant pathogens and strains of industrial significance that had been removed from the core NCTC holding and passed to other collections or discarded. Dr. Owen devoted a lot of time working on the catalogue as did other scientific staff. It was a huge job in pre-computer days, although entries could be recorded on a 'Flexowriter' so that successive editions would not require retyping of entries already made. Dr. Owen was particularly tasked with drawing up an alphabetical listing with up-to-date nomenclature (there were a lot of *Bacillus* spp. in the old lists) and then compiling the numerical list to go at the end of the catalogue. There was much updating of individual strain entries in a standard format with the name details and type strain status and history and references to add as well. Checking the proofs from the printers was also a big job and then re-checking to ensure any corrections had been correctly incorporated (all those *Salmonella* serotypes proved a particular nightmare). This new edition, the fifth, was published in 1972. The next edition followed in 1983: this proved an easier task but there were a lot of additions and deletions. Also the correct type strains and name changes had to be incorporated as the nomenclature needed to be consistent with the Approved Lists of Bacterial Names that had then just been published. The updating of the catalogue was an on-going task with the constantly developing nomenclature and new accessions being added. The seventh edition in 1989 included a new section on plasmids plus the NCPF holdings and the final hard copy catalogue in 1994 listed the NCTC cultures and plasmids, *Mycoplasma* cultures and specific antisera, *Leptospira* cultures and the NCPF cultures.

During the 1980s, it was appreciated that searching for key facts relating to microorganisms was an increasingly important aspect of biotechnology research. Apart from the drudgery involved, the task could be haphazard. The UK government sought to address this by providing a means of obtaining such information from remote computer terminals. Known as MiCIS, an acronym for Microbial Culture Information Service, the new service would enable subscribers to search its databank rapidly for information on microorganisms, or to pinpoint organisms with certain properties. MiCIS was to be developed by the Laboratory of the Government Chemist (LGC), an arm of the then Department of Trade and Industry. It would incorporate data on more than 30,000 microbial strains isolated, characterised, and stored largely by the nine national culture collections located in various parts of the UK. It was envisaged as the forerunner of a Europe-wide computer database system designed along similar lines, and eventually of a global service. It would become the first service of its kind to go live in Europe. However, before it could start, not all UK collections had their catalogue and strain data in an electronic form, including the NCTC. LGC therefore assigned two members of staff to visit each collection in turn to code up data from hard copy records into a common electronic format. Although the task was completed and the service commenced operations in March 1987, the culture

collections themselves did not use the database, so it was not kept current and eventually the service fell into disuse. In the late 1980s, international initiatives were also progressing between collections which had electronic catalogues of their collection holdings and associated data. One early initiative was the EEC sponsored Microbial Information Network Europe (MINE) which it was hoped would be fully operational by 1989. In 1996, the EU funded an initiative Common Access to Biological Resources and Information (CABRI) which developed into an online service where users could search a number of European Biological Resource Centre catalogues. These catalogues could be searched independently, or as one, and the located materials ordered online or by post; constituent collections include CABI, ECACC and NCIMB. The lack of an electronic database of the NCTC culture data precluded the NCTC from taking part in such initiatives.

With its new, more commercial, remit from October 1994, the NCTC began designing and producing sales literature and a pop-up stand to attend commercial exhibitions at international conferences. What it needed desperately though was an electronic database of the NCTC culture data. The author discovered that the original MiCIS data still survived at the DSMZ. They kindly provided the NCTC data. The NCTC signed up to the BioloMICS database which had been developed specifically for culture collections. Vincent Robert, who developed the software, took the NCTC data, ran it against the DSMZ database to collect all the equivalent numbers of each NCTC culture in other culture collections, then ran these numbers against other databases to draw in 16S, 23S ribosomal RNA and *gyr*B gene sequences. This at once gave the NCTC the electronic database it needed and from a scientific point of view was likely to be the best the NCTC would have for the foreseeable future. Cultures could be ordered directly through the BioloMICS database, but its drawback was a lack of compatibility with the HPA corporate 'ORACLE' finance system. Stocks of the NCTC cultures were maintained on a stand-alone 'SAGE' system; on this it was easy to manage the stock flow with issues/sales invoices and calculate an accurate closing stock within SAGE; there was uncertainty as to whether or not the stock movements could be captured in the 'ORACLE' system. Thus whilst sales invoices were generated by 'SAGE', the information on them had to be manually uploaded on a monthly basis into the 'ORACLE' sales ledger system so as to ensure the sales income was captured within 'ORACLE'. Within HPACC a new financial system was developed for sales from all four constituent collections. Once this came into operation and the NCTC invoicing was transferred to Porton, the stocks were migrated to the 'ORACLE' system with automatic sales invoices being generated in 'ORACLE'. Use of the BioloMICS database was then discontinued.

Other NCTC developments post-1994

With the advent of molecular methods, divergence had been reported between cultures of nominally the same strain held in different culture collections. Concerned by this possibility, as each new batch of NCTC cultures had been prepared from the previous batch, from 1996 and for the more popular cultures, seed stocks were prepared from the earliest available freeze-dried batches. Seed stocks of the NCTC cultures were preserved in liquid nitrogen on coated glass beads in polypropylene vials (Hazard Group 2 cultures only). These cryopreserved seed stocks were generally prepared from ampoule cultures opened in preparation for batch re-drying. Since these seed stocks would ultimately be used up, for particularly popular cultures seed stocks were also prepared in ampoules to be used for the creation of future cryopreserved seed stocks.

Freeze-dried cultures were relatively easy to handle and post. Restrictions on hazardous bacteria in the postal system were not introduced until the Vienna Convention (1964). In the NCTC in the 1970s cultures for accession or identification were often received inadequately packed and it was no surprise that packaging eventually had to meet internationally recognised standards, which became even more stringent from 1st January 2005, with only micro-organisms considered as diagnostic specimens accepted in the post. Not only was there a requirement to meet these new regulations in order to send cultures, but the increasing threat of hazardous pathogens falling into the wrong hands led to a whole raft of new legislation to be followed [Specified Animal Pathogens Order (SAPO) 1998; Specified Animal Pathogens (Amendment) (England) Order 2006; Anti-Terrorism, Crime and Security Act 2001; The Schedule 5 to the Anti-terrorism, Crime and Security Act 2001 (Modification) Order 2007; The Part 7 of the Anti-terrorism, Crime and Security Act 2001 (Extension to Animal Pathogens) Order 2007; The Biological Agents and Genetically Modified Organisms (Contained Use) Regulations 2010; Export Control Act 2002]. As in other countries, facilities holding hazardous pathogens had to be registered, individual transfers to be controlled and cultures supplied only from and to registered facilities.

Although the NCTC had experimented with other methods of preservation, lyophilised cultures in glass ampoules under vacuum were the ideal for culture collections because of the longevity of the cultures (one example at a 55-year count showed no drop in viability). Other methods enabled cultures to survive for only a year or so but were suitable for quality control use or (as the NCTC had initiated. *vide supra*) for use in quality control distributions. Dr. Arthur Codd, at the then Newcastle Public Health Laboratory

developed the short-term preservation of microbial cultures in the format of discs comprising bacteria or fungi in a solid water soluble matrix. These LENTICULE discs were produced in Newcastle and were quantitative, containing viable microorganisms in a certified and narrow defined quantity and were sold directly, abroad as well as in the UK. The NCTC produced a range of qualitative cultures in LENTICULE disc format. The NCTC was also supplied with them in quantitative format by Dr. Codd for resale. They were also employed in quality control distributions by the Food and Environmental Proficiency Testing Unit, who also developed a range in the quantitative format as certified reference materials which were made available to scheme participants. All these producers used NCTC and NCPF cultures. Once HPACC was established, the NCTC stopped supplying LENTICULE discs whilst Newcastle ceased supplying them directly and supplied them to the HPACC warehouse at Porton for onward distribution along with other HPACC products.

Molecular Identification Services Unit (MISU)

Although NCTC research came to an end in 1994 and the identification services had been re-located elsewhere in Colindale, there was a resurgence of research in June 1997 when Dr. Haroun N. Shah was appointed to head and initiate a new "Molecular Identification Services Unit" as part of the NCTC. MISU's remit was to analyse and report on "atypical, rarely isolated and emerging pathogens" so it began to explore, develop and implement new techniques that would provide new information other than the physiological tests that had long been the mainstay of microbiology laboratories. These new approaches might then find application in other laboratories. Initially, there were only two other staff; one of these, Dunstan Rajendram, was the recipient of the PHLS's first MRC PhD studentship. The analysis of long-chained cellular fatty acids using capillary-gas chromatography was initiated in 1998 through a capital equipment grant and was maintained until 2008, utilising NCTC strains to develop and validate the system.

In 1998, the laboratory began to isolate DNA and worked on several platforms in collaboration with various companies such as Applied Biosystems, MWG-Biotech, Li-Cor, Perkin Elmer, Pharmacia, GE-Healthcare and Roche to begin sequencing 16S rRNA. While 16S rRNA was used for studies on microbial evolution, its use as a diagnostic test was not done before. Sequencing was initially contracted through Cambridge Biosciences, while Blast searches were done in MISU. Eventually the method was confidentially applied. A new laboratory (not in NCTC) was established under Prof. Saheer Gharbia: the Genomics, Proteomics and Bioinformatics Unit (GPBU) in 2003 and DNA sequencing was established in Colindale. PCR products of isolates sent into MISU could now be sequenced in GPBU. The system worked efficiently, and in 2005, MISU became the first laboratory globally to achieve Clinical Pathology Accreditation to use 16S rRNA as a diagnostic tool.

In 1998, MISU also began to explore the potential of a very new and novel technology – Matrix-Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry (MALDI-TOF MS), initially with the mass spectrometry company, Kratos Analytical and subsequently Micromass (later taken over by Waters). A method was published (Shah et al., 2000), followed by its value in distinguishing poorly classified anaerobes (Shah et al., 2002) then publication of the first ever MALDI-TOF MS database (Keys et al., 2004) using NCTC strains, all of which had a huge impact in the field.

While GPBU continued to expand and consolidate in genomics, so much of the proteomics were being done in MISU that GPBU changed its name to Applied and Functional Genomics (AFGU) to expand its role in genome sequencing. MISU stayed part of the NCTC until 2008 at which time it was amalgamated with AFGU as the Department for Bioanalysis and Horizon Technologies (DBHT). In November 2011, the HPA agreed to implement MALDI-TOF MS as a diagnostic tool in Colindale. Instruments were soon purchased for several other HPA laboratories in preparation for implementation during the London 2012 Olympics, if required. Currently, MALDI-TOF MS is used globally and is regarded as a 'quantum leap for diagnostic microbiology' and systematics. It was pioneered initially using NCTC strains and two books (Shah and Gharbia 2010, 2017) were subsequently published to highlight the extensive work done to drive the success of this technology into microbiology and also current and future perspectives.

From October 1998, MISU and AFGU (DBHT) organised annual international conferences in genomics and proteomics. They were organised to showcase developing technologies in these fields and to ensure that HPA was involved in its development. These conferences generated significant income and additionally, considerable funding was obtained both by MISU and AFGU in the form of several major grants to support research.

Retirement

On 1st April 2012, the Head of NCTC retired after 41 years at Colindale, marking the apparent end of the era of the NCTC at Colindale. In June 2012, all "13 x 6" strain record cards were moved from Colindale to NIBSC in their original cabinets (see Figure 9). Only a reserve culture store then remained in use at Colindale.

By this time the NCTC held over 5,000 cultures of medical, scientific and veterinary importance, over 100 mycoplasmas, over 500 plasmids, host strains, bacteriophages and transposons, the most popular fungal cultures from the NCPF collection, provided the services of an International Depository Authority for patent strains and offered freeze-drying services (a major undertaking being the freeze-drying of cultures of *Mycobacterium tuberculosis* to create the TDR Tuberculosis Strain Bank in Belgium [Vincent et al., 2012]). Financially, the collection had been successful, in 1994/95 covering 84% of its costs. Much of this cost recovery had been the result of substantial price increases. In 1994, all ampoules were priced at £38.00. Under HPACC a banding structure was introduced such that in 2012 cultures could be £65.00, £150.00, or £300.00. Increasing prices, however necessary to cover costs, does result in fewer ampoules being provided (in 1992/93 6,294 ampoules, in 1993/94 7,297, in 2009/10 3,384 and in 2010/11 3,566). Striking an appropriate balance is not easy.

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Table 1. The UK National Microbiological Collections

CABI	Centre for Agriculture and Biosciences International (filamentous fungi)
CCAPa	Culture Collection of Algae and Protozoa
NCIMBa	National Collections of Industrial and Marine Bacteria (also other environmental bacteria)
NCPPB	National Collection of Plant Pathogenic Bacteria
NCYC	National Collection of Yeast Cultures
ECACCb	European Collection of Animal Cell Cultures
NCPVb	National Collection of Pathogenic Viruses
NCPFb,c	National Collection of Pathogenic Fungi (medically important fungi)
NCTCb,c	National Collection of Type Cultures (medically important bacteria)

a Separate collections at one time
b Consolidated within the Health Protection Agency Culture Collections (HPACC).
c Originally in PHLS.
ECACC, NCPV and NCTC were all planned to be co-located at Porton during 2012.

Table 2. Roles of NCTC staff contributing to the International Committee on Systematics of Prokaryotes (and its forerunners)

Subcommittee for the Taxonomy of Aerobic Bacteroidetes [formerly Flavobacterium- and Cytophaga-like Bacteria] (R. J. Owen, B. Holmes)
Subcommittee on Taxonomy of Aeromonadaceae, Vibrionaceae and related organisms (B. Holmes)
Subcommittee on the Taxonomy of Campylobacter and related bacteria (R. J. Owen, S. L. W. On)
Subcommittee on the Taxonomy of Enterobacteriaceae (R. St. John-Brooks, B. Holmes)
Subcommittee on the Taxonomy of Gram-negative Anaerobic Rods (Haroun N. Shah)
Subcommittee on the Taxonomy of Micrococci and Staphylococci (Roland Hill)
Subcommittee on the Taxonomy of Mollicutes (Ronald Leach)
Subcommittee on Numerical Taxonomy (Roland Hill)

Figure 1. The NCTC at the Lister Institute, Chelsea 1920-1939

Figure 2. The NCTC at the Lister Institute, Elstree 1939-1949

Figure 3. NCTC staff at NIBSC 2011 with one of two new secondary driers

Figure 4. NCTC relocates to NIBSC 2011 – Redundant secondary drier left at Colindale together with Barry Holmes, Head of NCTC 1999-2011

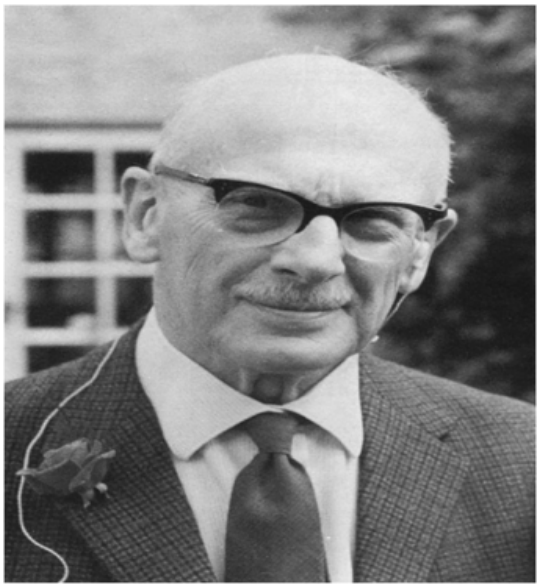
Figure 5. Head of NCTC ascends to summit of Mount Fuji with staff members of JCM

Figure 6. NCTC staff *circa* 1990

Figure 7. NCTC staff *circa* 2000

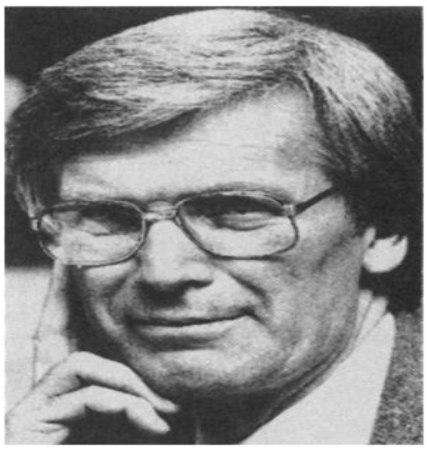
Figure 8. Curators of the NCTC

1920-1946 R. St John-Brooks



1947-1965 Samuel Tertius Cowan

1965-1978 Stephen Paget Lapage



1978-1994 Leslie Roland Hill

1995-1999 Menelaos Costas

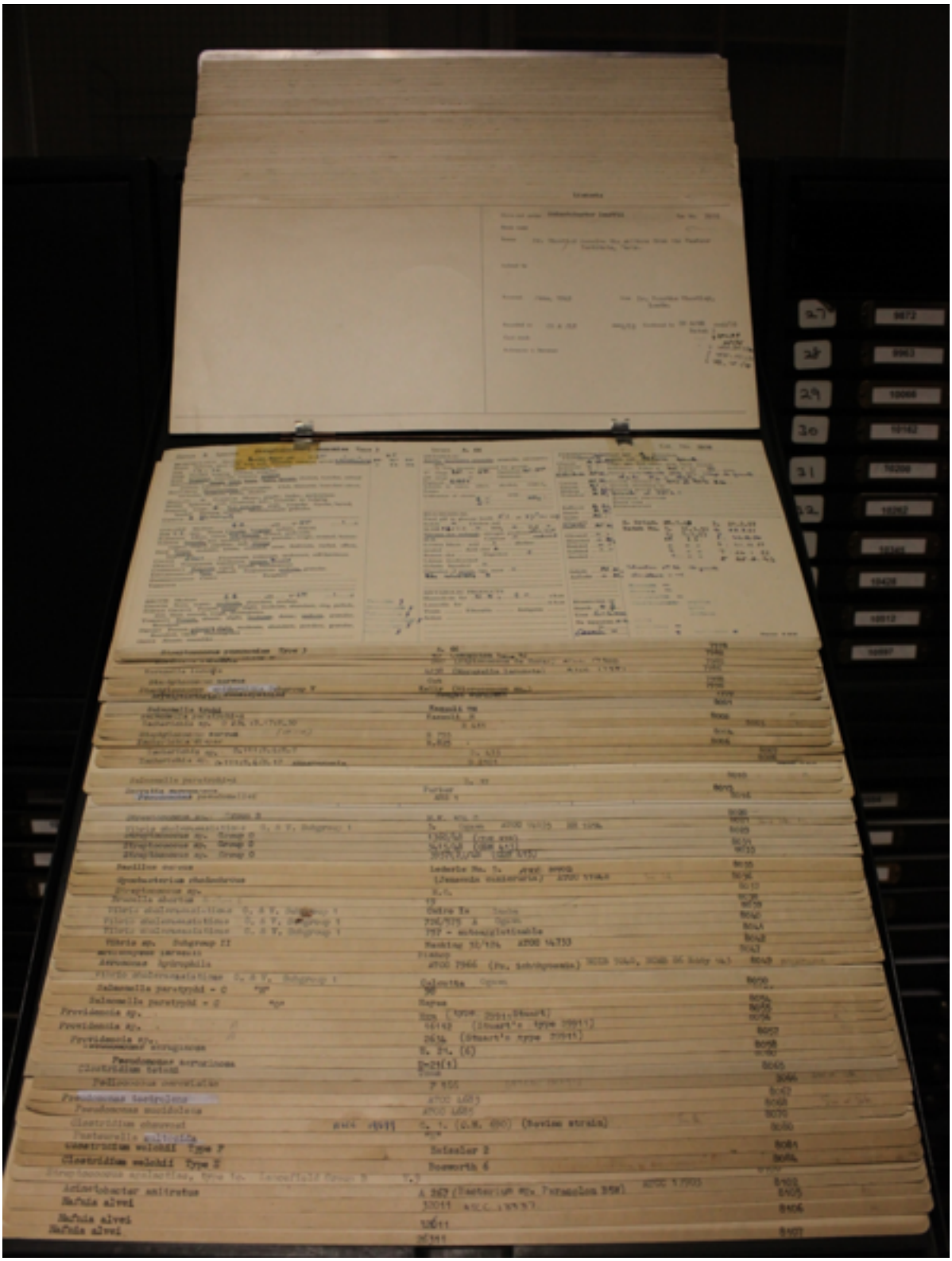


Figure 9. 13x6 cards in original cabinets

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Importance of Microbial Culture Collection in Pakistan: Challenges and Opportunities

Iftikhar Ahmed[1], Saira Abbas[2] and Hamza Tariq[1]

The importance of microbes in biotechnological, agricultural and industrial applications has inspired many Institutes to explore indigenous microbial diversity from various ecological niches of Pakistan. Among these, the most common examples are the strains associated with plant growth-promoting activities that could be used as biofertilizers (Abbas et al. 2015a; Ahmed et al. 2014c; Bangash et al. 2015; Hayat et al. 2010); strains for bio-remediation of heavy-metals polluted soils/water systems (Abbas et al. 2015a; Abbas et al. 2014; Abbas et al. 2015b); strains useful in food processing industry (Schmidt et al. 2012); pathogenic strains responsible for the bacterial blight of rice (Nagarajkumar et al. 2004) and citrus canker; and other extremophilic (i.e. NaCl tolerant, heavy-metals tolerant) strains (Abbas et al. 2015a; Abbas et al. 2014; Abbas et al. 2015b; Bangash et al. 2015; Jamil et al. 2013; Roohi et al. 2012; Roohi et al. 2014a; Roohi et al. 2014b) for novel biotechnological applications. However, very few of these microbes have been confirmed at the species level based upon 16S rRNA gene sequence (Abbas et al. 2015a; Abbas et al. 2014; Abbas et al. 2015b; Ahmed et al. 2014a; Ahmed et al. 2014b; Ahmed et al. 2014c; Ahmed et al. 2014d; Amin et al. 2016a; Amin et al. 2016b; Amin et al. 2016c; Amin et al. 2018; Amin et al. 2017; Bangash et al. 2015) and many have been incorrectly identified being published based on morphological data alone (Fatima et al. 2009; Hasan et al. 2009; Ikram-ul-Haq et al. 2009). Most of the strains collected by various labs were either stocked in glycerol as unidentified strains or discarded over time due to contamination or loss of interest of researchers at end of the particular projects. There are many examples within various life sciences departments of Pakistani Universities or research organizations, where no old authentic isolated and purified strains have been retained. Despite tireless efforts of researchers from various institutes, new bacterial species are rarely published from Pakistan, except those reported recently by our group at National Agricultural Research Centre (NARC), Islamabad in addition to few others (Imran et al. 2010; Khalid et al. 2015). Major reasons include a lack of facilities and specialized equipment for chemotaxonomic analyses as well as expertise required for the validation process of novel species. There is a big challenge to validly publish any novel species from Pakistani ecology because only few efforts have been made systematically to recognize the strains at national and/or international level based upon scientific background and molecular tagging (gene sequence).

Microorganisms are classified in risk groups signifying their safety level; only strains from group risk 1 are considered as safe and can be used for product development. If the process of identification is not performed with the latest methods, it may be possible that any pathogenic strains may be missed and become part of the developed bio product that belongs to risk group 2 or higher. Since any environment / ecology is a natural reservoir for human opportunistic pathogens, the taxonomical identification of the beneficial isolates must be performed at an early stage in the selection process during development of any bio-product. This must be performed using the best available technology, for example the sequencing of conserved markers such as 16S rRNA, 23S rRNA etc. Indeed, microbial strains should be identified by molecular biological methods, mainly at DNA level, which allows the identification of organisms and the delineaton of said organisms to the correct risk groups and recognize opportunistic pathogens. Contamination in microbial culture is also another common problem, thus, identification of microbial strains to be used in any product development which is going to be commercialized, its quality control should be managed over time with some standards. It's a matter of fact that commercialized product should be registered or patented so identification of beneficial microbes should also be a prerequisite to patent or register any kind of bio product such as biofertilizer or microbial process. The identification process involves the use of partial sequence analysis of the 16S rRNA gene besides a basic characterization, the analysis of the cellular fatty acids, differentiating classical physiological and biochemical tests and/or ribotyping analysis etc. Thus, a taxonomically unassigned strain needs to be identified and characterized by a wide range of approaches

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to obtain a broad range of informative data from the genetic and epigenetic level, including morphology, physiology, chemistry, DNA patterns and sequences, and whole genome hybridization.

Microbial Culture Collections play an important role in offering services not only to preserve microorganisms using techniques that maintain viability, purity, and important characteristics of microorganisms, but also to supply high quality microorganisms for use in teaching, research and industrial applications. In order to meet modern demands for further advancement of bio-economy, a concept of bio-repository of biological materials and information in Pakistan has been introduced recently: Pakistan Bio-Resources Centre (Pak-BRC) for conservation of bio-assets of Pakistan. Pak-BRC is considered to be one of the key scientific infrastructures to support agriculture sector, the health sector and the industrial sector or other sectors, and in turn ensure that these advances help to drive economic growth at desired pace in the subjects of biotechnology, agriculture and life sciences industry. Thus, Pak-BRC is fundamental to harnessing and preserving our microbial biodiversity and serve as an essential element of the infrastructure for research and development. Pak-BRC is also necessary to protect the intellectual property rights of microbial assets of country through management of data bases and DNA tagging the biological material using molecular techniques. Consequently, it will improve usage of microbial materials while facilitating collaborative research and development programs without duplication of effort at the national level.

In Pakistan, extensive research work has been conducted on microflora and continues. However, only few laboratories in Research Institutes and Universities have maintained microbes for their specific research purposes such as NARC, Islamabad, University of Agriculture and NIBGE, Faisalabad; Punjab University, Lahore, etc.. Long term preservation of microbes is a neglected process in Pakistan. According to World Data Centre for Microorganisms (WDCM), there have been following five Culture Collection Centres registered from Pakistan (WFCC, 2018; http://www.wfcc.info/ccinfo) (Table 1).

Among these, PTCC and NCCP deal with bacteria, whereas ESBL-Pak mostly deals with pathogenic microbes, while other two mainly involved in the collection of fungus strains. However, since the establishment of PTCC for the last two decades, none of the strain has been characterized or validated at species level. There are 15,625 species of bacteria (as of May 5 2017) identified globally (LPSN, www.bacterio.net), but from a rich ecolosystem of Pakistan, but to date, only 27 species have been reported, validated and published (Abbas et al. 2015a; Abbas et al. 2014; Abbas et al. 2015b; Ahmed et al. 2014a; Ahmed et al. 2014c; Amin et al. 2016a; Amin et al. 2016b; Amin et al. 2016c; Amin et al. 2018; Amin et al. 2017; Bangash et al. 2015; Roohi et al. 2014b). In Pakistan due to diverse nature and due to limited taxonomic research on identification of microbial strains, there is a huge potential to explore our own microbial assets and identify novel species. Microbiota of any region or ecology may be specific to that environment, thus exploring the microbiota of Pakistan may furnish some incredibly useful and rarely explored taxa / strains that may revolutionize the biotech industry and be important in other applications.

Recently, Pakistan Agricultural Research Council (PARC) took an initiative to improve identification and preservation of economically important bacterial strains from Pakistani ecosystems. In this regard, PARC established microbial bioresources repository: National Culture Collection of Pakistan (NCCP) for preservation of this bio-asset of Pakistan. During our studies for collection and preservation of indigenous beneficial strains many promising novel candidate strains were identified based upon 16S rRNA gene sequencing, which can be delineated as novel species. However, to meet the minimum standards to describe any novel species of bacteria, many chemotaxonomic experiments need specialized equipment not available in the majority of laboratories in Pakistan. This NCCP project was initiated in 2009 and later, a complete project that included the important infrastructure was approved in 2015 by the Ministry of National Food Security and Research. But unfortunately, no resources were released to NCCP for the development of resources.

Limited lab facilities and meager resources led to the establishment of international collaborative links with Japan, China and Korea for delineation of our candidate strains as novel species. Now, for the first time in the history of Pakistani research, these productive collaborations have made it possible to validly publish the following novel species of bacteria from Pakistani ecosystems:

- Novel genus *Caldovatus sediminis* gen. nov., sp. nov. (validated 2017)
- *Tepidimonas alkaliphilus* sp. nov., (published 2017 and validated 2018)
- *Tepidimonas sediminis* sp. nov. (published 2017 and validated 2018)
- *Phenylobacterium tarrae* sp. nov., (published and validated 2018)

Continued on next page

- ◊ *Nocardioides thalensis* sp. nov., (validated 2017)
- ◊ *Thermus caldifontis* sp. nov., (validated 2017)
- ◊ *Streptomyces caldifontis* sp. nov., (validated 2017)
- ◊ *Microvirga pakistanensis* sp. nov., (published 2016, validated 2017)
- ◊ *Nocardioides pakistanensis* sp. nov., (validated 2016)
- ◊ *Bacillus malikii* sp. nov. (validated 2016)
- ◊ *Alcaligenes pakistanensis* sp. nov., (validated 2016)
- ◊ *Kushneria pakistanensis* sp. nov., (validated 2015)
- ◊ *Acinetobacter pakistanensis* sp. nov., (validated 2015)
- ◊ *Deinococcus citri* sp. nov., (validated 2014)
- ◊ *Lysinibacillus composti* sp. nov., (validated 2014)
- ◊ *Lysinibacillus pakistanensis* sp. nov., (validated 2014)
- ◊ *Bacillus pakistanensis* sp. nov., (validated 2014)
- ◊ *Sphingobacterium pakistanensis* sp. nov., (validated 2014)
- ◊ *Cellulomonas pakistanensis* sp. nov., (validated 2014)

Since, identification and systematics of bacteria have not been extensively included in the main applied microbiological research work in Pakistan, the rich unexplored ecology of Pakistan may offer an opportunity to explore many additional novel species of bacteria by the application of polyphasic taxonomic characterization and their preservation in the National Culture Collection of Pakistan (NCCP). The emphasis will be focused on the addition of novel beneficial microorganisms useful in agriculture and industry. There is a growing need to collect many more strains from various facilities for identification and preservation in NCCP.

The major gaols of NCCP would be:

I. A large number of economically important bacteria will be identified using molecular techniques and these will be available to biotechnology and industry. The identified (and characterized) strains will be utilized as plant growth-promoters and can be commercially made available to the farming community.

II. A large number of economically important strains preserved in NCCP as a resource for future scientific and academic research.

III. Changing climate conditions affects microbial diversity and may result in loss of many beneficial strains. NCCP will help to protect and restore these endangered microrganisms as a global resource.

IV. Human Resource development would be another output, as researchers of NCCP and students involved in these studies would be trained and work in collaboration with other Institutes on identification and chemotexonomic characterization of novel strains as a new species. Thus, a highly trained workforce would be established within Pakistan.

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Table 1. Culture Collection Centres registered in Pakistan

Name of Microbial Culture Collection	**Location**	**Established since**	**Algae**	**Bacteria**	**Fungi**	**Yeast**	**New Species reported from Pakistan**
Pakistan Type Culture Collections (PTCC)	PCSIR Labs, Lahore	1992	20	100	60	33	0
National Fungal Culture Collection of Pakistan (NFCCP)	University of Agriculture, Faisalabad	2003	0	0	125	0	0
First Fungal Culture Collection of Pakistan (FCBP)	University of Punjab, Lahore	2004	0	423	1260	0	0
National Culture Collection of Pakistan (NCCP)	**NARC, Islamabad**	**2010**	**0**	**1463**	**0**	**0**	**27**
Extended Spectrum Beta Lactamase-Pakistan-121 (ESBL-Pak-121)	Infection Control Society, Karachi	2012	0	27	7	0	0

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Halophilic Archaea from saline environments in India

Pradnya P. Kanekar and Snehal O. Kulkarni

Abstract

Halophilic archaea are salt loving microorganisms that usually inhabit hypersaline environments like solar salterns, salt lakes, salt deposits, salt crystals etc. and rarely from low saline environments like sea water. India is rich in microbial diversity. The coastal regions so far explored for isolation of haloarchaea include Rajasthan, Gujarat, Maharashtra, Goa and Tamil Nadu, salt lake of Rajasthan, soda lake of Lonar, North Maharashtra, Andaman Islands. Haloarchaea from these regions have also been isolated and identified. Haloarchaeal genera namely *Natronobacterium, Natronococcus, Haloferax, Halobacterium, Natrinema, Haloarcula, Halorubrum, Halogeometricum, Halococcus, Halolamina, Halosarcina, Halostagnicola, Haloterrigena, Natrialba, Natronoarchaeum, Haloadaptatus and Chromohalobacter* have been described.

The beauty of our mother earth lies in its diversity of living species including microorganisms. Geological activities have created a number of habitats that have shaped the evolution of microorganism leading to the establishment of a high diversity of different organisms. Some of these environments exhibit extreme conditions e.g. high temperature in thermal springs, high salinity and alkalinity in soda lakes, high salinity in salt deposits etc. Man made activities also lead to the formation of extreme environments e.g. acid mine drainage, solar saltpans, cement manufacturing, potato process waters etc. Microorganisms that survive and live in such extreme environments are termed as extremophiles. Halophiles are one of these groups of organisms that thrive in saline environments. Compared to thermophilic, acidophilic and alkaliphilic microorganisms, less documentation has been made in case of halophilic microorganisms.

Halophilic microorganisms inhabit saline environments such salt lakes, salt deposits, solar salterns, salt pans, saline ponds, etc. Depending upon their requirement of salt for growth, they are classified as slight halophile (0.2-0.5 M NaCl), moderate halophile (0.5-2.5 M NaCl), borderline extreme halophile (1.5-4.0 M NaCl) and extreme halophile (> 2.5 M NaCl). Halotolerant organisms do not require NaCl for growth but can tolerate high content of salt (0.0 to 5.2 M NaCl) (Grant et al., 2001). The haloarchaea are classified under the domain *Archaea*, phylum *Euryarchaeota*, class *Halobacteria*, orders *Halobacteriales, Haloferacales* and *Natrialbales*, families *Halobacteriaceae, Haloarculaceae, Halococcaceae, Haloferacaceae, Halorubraceae* and *Natrialbaceae* (Gupta et al., 2015, 2016)

For isolation of halophilic archaea, different nutrient media with high concentration (3-4 M) NaCl have been devised e.g. Sehgal and Gibbons (SG) medium (Sehgal and Gibbons, 1960); Tindall medium (Tindall and Truper, 1986) etc. Because of the high concentrations of NaCl in the nutrient media, selective isolation of halophilic archaea and elimination of halotolerant microorganisms is achieved. Characteristic features of halophilic archaea include typical pink red/ orange red coloured colonies, pleomorphic nature of cells, requirement of 1.5 M NaCl for growth, ether lipids in cell membrane, resistance to antibiotic chloramphenicol, inhibition of growth by sodium taurocholate, requirement of Mg2+, amino acids for growth in case of some species and more importantly lysis of cells in water and low saline solution. Globally, haloarchaea have been reported from different saline environments. A number of authros have reviewed these organisms; Rodriguez-Valera et al. (1979) reported isolation of extreme halophiles from low saline environment i.e. seawater. The Dead Sea, a terminal lake at the border between Israel and Jordon, and the Great Salt Lake, Utah, USA hasve been extensively studied for 20 years for isolation of haloarchaeal strains. Oren et al. (1990) reported the isolation of novel haloarchaeal species viz. *Halobacterium gomorrense*, *Haloarcula marismortui*, *Haloferax volcanii* from Dead Sea. Oren et al (2000) also reviewed isolation of novel haloarchaeal species from various eco niches such as salt pans, solar salts, crystallizer ponds of solar salterns, hypersaline lakes, salt mines as well as hypersaline soda lakes. Presence of halophilic microorganisms and haloarchaea from ancient rock salt and salt sediments have been reported by several researchers viz. (Stan-Lotter et al., 1999, 2002) as reviewed by Grant et al., (1998) and McGenity et al., (2000). Kanekar et al. (2012) also reviewed taxonomy, diversity and physiology of halophilic microorganisms including halophilic archaea.

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India is a country endowed with rich biodiversity including of microorganisms isolated from natural saline environments include Sambhar salt lake in Rajasthan (Fig. 1), Great and Little Rann of Kutch, Gujarat, soda lake of Lonar, Buldhana District, North Mahatrashtra, low saline coastal sediments, sand and sea water of Arabian sea across West Coast of India. Man-made habitats include mainly solar salterns from Mumbai, Goa, Kandla Port, Gujarat, Kanyakumari, Tamilnadu, Shiroda, Sindhudurga District, etc. The West coast of India popularly known as Western Ghats are recognized as hot spots of Biodiversity. This coastal region is spread over from Gujarat to Tamilnadu in South India through Maharashtra, Goa and Kerala and explored for halophilic biodiversity.

Figure 1. Sambhar Lake Rajasthan showing pink red bloom of haloarchaea

The first report of isolation of haloarchaea from India was from Sambhar salt lake, Rajasthan. The lake is both saline and alkaline and situated at 26° 58 'N and 75° 5'E in the middle of a closed depression in the Aravalli mountain ranges, approximately 65 Km northwest of Jaipur. Upasani and Desai (1990) reported chemical composition of brines of Sambhar lake and isolated six strains of haloalkaliphilic archaea from brines taken from the main lake as well as solar evaporation pans of Sambhar lake. The salt content of the brines ranged from 12-30 % and pH 9.5-10.5. The authors used modified Brown medium having pH 9.5 and varied the content of NaCl and KCl. The strains were studied for their lipid profile and found to contain phosphatidylglycerophosphate (PGP), phosphatidylglycerol (PG), and phosphatidic acid (PA).The haloarchaeal strains were identified as *Natronobacterium* and *Natronococcus*. *Natronobacterium* strains SSL 1 and SSL6 isolated from Sambhar Lake were found to contain novel glycolipid.

Gujarat coastal region has been extensively explored for halophilic microorganisms, the major salt producing sites in Gujarat are Saurashtra at Kandala, Jamnagar, Maliya, Mithapur, Porbander, and Bhavnagar which become the natural habitats for haloarchaea. Haloarchaea have been isolated from marine salterns at Mithapur, Gujarat (Upasani et al., 1994). The organisms were identified as *Haloferax* MSW1 (NCMB 2287) and *Halobacterium* MSW 5 (NCMB 2288). These along with the other haloarchaeal strains were studied for their lipids. Dave and Desai (2006) studied the microbial diversity of marine salterns near Bhavnagar. The sample sites i.e. marine salterns are located at 22° 10' N and 72° 15 'E on the Western and Northern coasts of the Gulf of Khambhat, Arebian Sea, about 25-30 Km north of Bhavnagar. Out of 73 selected isolates of halophiles, six isolates were found to be highly pleomorphic and halophilic. They were identified as *Natrinema thermotolerant*. Halophilic archaebacterium have been isolated from salt pan situated in Kandla and identified as *Halobacterium* sp. (NCBI Gen Bank Accession No. DQ465014) (Akolkar et al.,2008).

Thomas et al (2012) investigated the distribution of haloarchaea in hypersaline marshy environment of Little and Great Rann of Kutch, Gujarat. The region covered for collection of soil and water samples lies between 23°10.727'N, 070°43.733'E of Little Rann and 23°57.7215'N, 069°43.7611'E of Great Rann of Kutch. The haloarchaeal population was represented by the genera *Halobacterium, Natrinema, Haloferax, Haloarcula, Halorubrum, Halogeometricum, Halomicrobium,* and some uncultured haloarchaeal clones. Phosphate solubilizing haloarchaea isolated from Rann of Kutch, Gujarat included genera *Haloarcula, Halobacterium, Halococcus, Haloferax, Halolamina, Halosarcina, Halostagnicola, Haloterrigena, Natrialba, Natrinema* and *Natronoarchaeum*.

The coastal region of Western Maharashtra is popular for tourist destinations such as Mumbai Fort, Gate Way of India, Shreewardhan and Harihareshwar of Raigad District, Sindhudurga Fort, Rock Garden Malvan, Deobag, Tarkarli of Sindhudurga District, Bhagavati port of Ratnagiri, Thiba palace of Ratnagiri District and many other places. A number of solar salt pans are also in use. e.g. Mulund, Bhandup, Bhainder of Mumbai, Shiroda of Sindhudurga District, etc. All these saline environments are populated by halophiles but comparatively less explored. Phylogenetic analysis of archaeal Ribosomal DNA sequences from salt pan sediment of Mumbai have been documented (Ahmad et al., 2008). Upper sediment (2-10 cm distant from surface) samples were collected from salt pans of Mumbai, located geographically between 18°53'Nand 19°20'N latitude and 72°45'E and 73°00'E longitude. Total genomic DNA was extracted from samples and PCR amplification of 16S rRNA genes was performed using archaea specific primers. Cloning of environmental PCR products was carried out followed by Restriction Fragment Length Polymorphism (RFLP) screening of rDNA clones and nucleotide sequencing of cloned SSU rRNA genes. Phylogenetic analysis of cloned SSU rRNA gene sequences was performed by comparing the closest database relatives of all sequences

generated to 16 S rRNA gene sequences available at NCBI. Based on the similarity values, the closest relatives among *Euryarchaeota* were found to be *Haloarcula amylolytica*, *Haladaptatus pauchihalophilus* and many uncultured *Archaea* including methanogenic species. Pathak and Sardar (2012) have isolated haloarchaea from solar saltern of Mulund (E) 19°10'12"N, 72°57'18''E, Mumbai. Based on biochemical and physiological characterization, the haloarchaeal strains were found to belong to genera *Chromohalobacter, Haloferax, Halobacterium* and *Halorubrum*.

Isolation of halophilic archaea from sea water and soil samples from salt production sites of Mumbai have been documented (Digaskar et al., 2015). The haloarchaeal genus *Haloferax* was found to be dominant in the saltern ecosystem. Thombre and Oke (2015) have reported the Studies on stress proteins induced by temperature stress in extremely halophilic archaeon *Haloferax mediterranei* RT 18 isolated from surface water samples of Marine Drive area of Mumbai. Soluble proteins were isolated, separated by SDS-PAGE and identified by MALDI-TOF MS-MS analysis. The stress protein identified as beta proteosomal subunit is found to play role in maintaining protein homeostatis.

Kanekar et al. (2015a) explored rock- pit sea water from Rock Garden, Malvan, Sindhudurga District, West Coast of Maharashtra for isolation of haloarchaea. Eleven haloarchaeal strains obtained were characterized and identified based on 16S rRNA gene sequencing as *Haloastagnicola larsenii*. This is presumably the first report of the isolation of *Halostagnicola larsenii* from Arabian sea water, West Coast of India. Kanekar et al. (2015b) have reported *Halostagnicola* sp. from sand and sea water of Andaman Islands. Kanekar et al. (2016a) have also investigated colonization of rocks of Rock Garden, Malvan, Sindhudurga District by the haloarchaea imparting red coloration to the rock, the organism was identified as *Haloferax larsenii*. This is presumably the first report of isolation of haloarchaea from red coloured rocks of rocky beach. Patil et al. (2016) have reported haloalkaliphilic archaea namely *Natrinema* sp., *Natrialba wudunaoensis*, *Natrialba chahannaoensis*, *Natronobacterium innermongoliae* from alkaline and saline Lonar Lake, North Maharashtra, India.

Goa is a coastal state (15°34'60 N, 74°0'E) and one of the beautiful tourist places of India having natural beaches enjoyed by the tourists. Both the sea water and salt pans in and around Goa have been explored for isolation of halophiles. Branganca and Furtado (2009) isolated and characterized haloarchaea from low salinity coastal sediments and waters of Goa. Water and sediment samples were collected from coastal regions of Goa namely Talpona, Vasco, Dona Paula, Miramur and Bainguinim. The haloarchaeal isolates obtained using nutrient media containing 20 % salt were tentatively identified as *Halobacterium* and *Haloarcula* sp. Mani et al (2012) reported isolation of halophilic archaea from natural solar saltern of Goa. Salt making is being practiced for many years in Goa. Salt pans are located in Pernem, Bardez, Tisawadi and Salcete talukas of Goa. The authors selected a single solar saltern at Ribandar (15°30'N, 73°51'E) to examine culturable haloarchaeal diversity. Water and sediment samples were collected 0-10 cm distance from surface twice during the two phases of salt production. In pre-salt harvesting phase /initial stage/salt dilute stage, the haloarchaeal strains of *Halococcus salifodinae* were obtained while in salt harvesting phase/ crystallization stage, haloarchaeal strains of *Halococcus salifodinae, Haloferax volcanii, Haloarcula argentinensis, Haloarcula japonica, Haloarcula* sp., *Halorubrum* sp., and *Haloferax alexandrinus* were obtained. The carotenoid pigment was extracted from the orange red growth of the isolates and spectrophotometrically confirmed as bacterioruberin. Metal tolerance of these haloarchaeal strains was investigated by Chaudhary et al. (2014). The strains of *Halococcus salifodinae, Haloferax volcanii, Haloarcula japonica* and *Halorubrum* sp. could grow and tolerate cadmium concentration ranging from 0.5-4 mM.

The peninsular coast of India particularly the Kanyakumari coast is the beautiful tourist place in India. Haloarchaea were isolated from saltern soil samples (K.R.T. Asha et al., 2005). There are over 5000 salterns in the coastal regions of Kanyakumari, thus making the site a congenial environment for growth of haloarchaea. Based on cultural, morphological and biochemical characterization, the haloarchaeal isolates were tentatively identified as *Haloarcula vallismortis* (HA3) and *Haloarcula quadrata* (HA9).

Manikandan et al. (2009) have studied microbial diversity of solar salterns of Tamil Nadu. Samples from the crystallizer ponds were collected from multipond solar salterns in Kelambakkam, Marakanam, Vedaranyam of Tamil Nadu. The haloarchaeal isolates belonged to *Haloferax* sp. *Haloferax larsenii*, *Halorubrum* sp., *Halogeometricum borinquense*, *Halobacterium* sp., *Haloarcula* sp., *Halorubrum sodomense*, *Halobacterium salinarum* and *Haloferax volcanii*. Thus the microbial community of Tamil Nadu salterns was found to be dominated by haloarchaeal genera *Haloferax* and *Halorubrum*. Haloarchaea were isolated from soil samples collected from salt pans of Kanyakumari. The strain BTSH 10 was identified as *Natrinema* sp. Among the various saline environments, rock salt has been looked upon as a source of haloarchaea by researchers over the globe. The presence of viable microorganisms from ancient rock salt was described by Reiser and Tasch (1960)

and Dombrowski (1963). Norton et al. (1993), Stan-Lotter et al.(1999), McGenity et al. (2000) ,Stan-Lotter et al. (2002) and Gruber et al. (2004) reported the presence of haloarchaeal strains of *Haloarcula, Halorubrum, Halococcus salifodinae, Halococcus dombrowskii* sp. nov., *Halobacterium noricense* from various rock salt deposits and mines (Fig.2). Black Salt is a type of rock salt commonly called as Himalayan Black salt. It is salty, pungent smelling condiment used in South Asia. The Black Salt contains sodium chloride, trace impurities of sodium sulphate, sodium bisulphate, sodium bisulphide, iron sulfide and hydrogen sulphide. Due to presence of iron sulphide, it forms brownish pink to dark violet translucent crystals when whole. When ground into powder, it looks light purple to pink in colour (Fig.3). Black salt is considered as a cooling spice in Indian Ayurvedic preparations and reported to help in relieving intestinal gas, heartburn, hysteria etc. (Ali, 1999 ; Sadhale and nene, 2004; Aggarwal and Kotwal,2009). Since there were many reports regarding occurrence of haloarchaeal strains in different rock salts and the commercial Indian black salt looks pink in colour, it was thought to be a source of haloarchaea. Kanekar et al (2016 b) have isolated *Halostagnicola larsenii* IBS (MCC 2956) from Indian Black salt procured from local market by enrichment of the sample in Sehgal and Gibbons (SG) medium (Sehgal and Gibbon, 1960) containing 3.42 M NaCl. Pure culture obtained from well isolated pink-red colony was identified based on morphological, physiological and biochemical characterization and 16 S rRNA gene sequencing (Fig. 4). This is first report of isolation of *Halostagnicola larsenii* from commercial Indian black salt. Earlier Henriet et al. (2014) reported occurrence of two haloarchaeal genera viz. *Halarchaeum acidiphilum* and *Halobacterium noricense* from Himalayan pink salt.

Figure 2. Rock salt mine in Himachal Pradesh, India

Figure 3. Indian Black salt showing purplish black crystals and pink powder

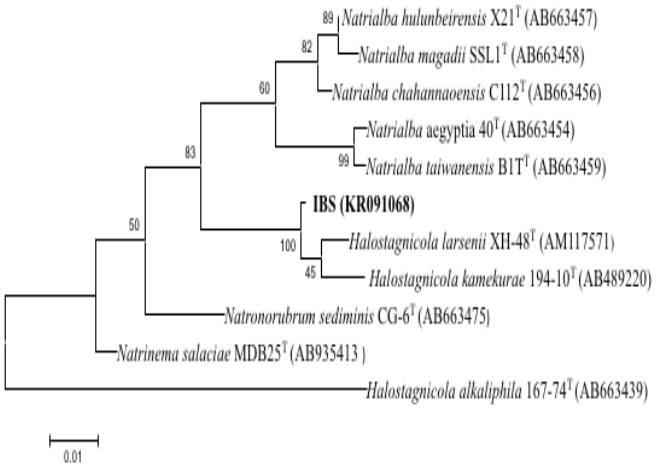

Figure 4. Neighbor-joining phylogenetic tree based on 16 S rRNA gene sequences indicating the relatedness of the strain IBS to *Halostagnicola larsenii* XH-48T and some haloarchaeal species. Accession numbers are shown in parentheses. Bootstrap values (%) are based on 1000 replicates. Bar represents expected changes per site.

Conclusion

Halophilic archaea are unique microorganisms endowed with the property to live and grow in high saline environments like salt lakes, solar salterns, salt deposits, ancient rock salt mines, salt crystals and rarely from low saline environments like sand and sea water. Moderate attempts have been made to study their biodiversity mainly from West Coast of India viz. coastal regions of Gujarat, Maharashtra, Goa and Tamil Nadu, salt lake of Rajasthan, soda lake of Lonar, North Maharashtra, Andaman Islands. Thus in closing it is apparent that a limited number of saline sites have been explored from India for isolation of haloarchaea and there are important opportunities to study biodiversity of halophiles from India.

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My life with the genus *Frankia*: an ongoing love affair

Imen Nouioui

Since my undergraduate days, I have been fascinated by the nature of microbial plant interactions and how they might be used to improve agricultural and environmental practices. I am especially interested in nitrogen-fixing actinobacteria belonging to the genus *Frankia* as they form mutualistic associations with dicotyledenous plants that might be used to stabilise sand dunes and decrease the spread of the Sahara Desert. Plants infected with *Frankia* are classified in over 200 dicotyledenous plants which represent 8 angiosperms families (Torrey and Tjepkema, 1979). These associations lead to the development of root nodules on host plants where the microsymbionts fix nitrogen for the benefit of the macrosymbionts (Magallon et al., 1999; Normand and Benson, 2012). The high rates of nitrogen-fixation achieved by actinorhizal plants allows them to colonise marginal soils thereby opening up the prospect of using such plants as pioneers in nitrogen-deficient soils, in land reclamation, as a source of timber and pulp (Benson and Silvester, 1993).

The ecological significance of actinorhizal association led to intensive studies on host plant ecology, mechanisms of infection and root nodule physiology (Benson and Silvester, 1993). In contrast, until recently, somewhat sporadic progress was made unravelling the subgeneric classification of *Frankia* strains mainly due to difficulties in isolating and characterising such slow-growing actinobacteria, problems compounded by the limited availability of representative strains from culture collections. These limitations meant that different *Frankia* strains were used in molecular systematic studies (Akimov et al., 1991; Akimov and Dobritsa, 1992; An et al., 1983; Bloom et al., 1989; Fernandez et al., 1989; Lumini and Bosco, 1996), including comparative analyses of conserved gene sequences (Murry et al., 1995 ; Bautista et al., 2011). Nevertheless, it became apparent from phylogenetic, morphological and physiological studies that the genus was markedly heterogeneous even though only a single species, *Frankia alni* (Woronin 1866; Von Tubeuf 1895) was recognised albeit without a recognised type strain. Furthermore, it also became clear from phylogenetic studies based on 16S rRNA (Normand et al., 1996), *gyr*B, *gln*II genes (Nouioui et al., 2011), 16S-23S rRNA internal transcribed spacers (Ghodhbane-Gtari et al., 2010), multi-locus gene sequences (*atp*1, *fts*Z, *dna*K, *gyr*A and *sec*) (Gtari et al., 2015) and core genome studies (Tisa et al., 2016) that most *Frankia* strains could be assigned to four clusters, the members of which showed distinct host ranges. Cluster I strains infect host plants belonging to the *Belutaceae* and *Casuarinaceae* (except *Gymnostoma*); those assigned to cluster 2 infect *Ceanothus* (*Rhamnaceae*), the subfamily *Dryadoideae* (*Rosaceae*), *Coriariaceae* and *Datiscaceae* while the more genetically diverse strains of cluster 3 are infective *Colletieae* (all actinorhizal *Rhamnaceae* except *Ceanothus*), *Myricaceae*, *Gymnostoma* and occasionally on *Alnus*. The remaining lineage encompasses "atypical" strains which are unable to re-infect actinorhizal host plants or form infective root nodules, and /or unable to fix nitrogen.

My PhD project was designed to caste light on the phylogeny and evolution of *Frankia* strains based on the anlaysis of *gln*II, *gyr*B, *nif*H and 16S-23S rRNA spacer sequences. It was evident from the resultant concatenated trees that the strains fell into four evolutionary lineages, as described above, which provided the basis for subsequent phylogenetic taxonomic studies. The variation of the average pairwise distance between strains assigned to the four clusters suggested that over evolutionary time *Frankia* had undergone a gradual shift from a saprophytic to a facultative and mutualistic lifestyle. In a corresponding study based on bacterial and plant gene sequences, it was shown that *Frankia* strains associated to *Coriaria* has evolved independently.

These critical developments opened up the possibility of undertaking comprehensive phylogenetic studies on *Frankia* strains to resolve outstanding taxonomic issues. In a seminal polyphasic taxonomic study (Nouioui et al., 2016), it was proposed that *Frankia alni* ACN14a, isolated in pure culture from *Alnus viridis* subspecies *crispa* with morphological properties matching the original description of *F. alni*, be recognised as the type strain of this species according to Rule 18f of the International Code of Nomenclature of Prokaryote. Two additional species were proposed for strains CcI3 and BMG5.12 isolated from *Casuarina cunninghamiana* and *Elaeagnus angustifolia*, namely *Frankia casuarinae* and *Frankia elaeagni*, respectively. These isolates, like the *F. alni* strain, were based on the results of chemotaxonomic and phenotypic studies and on complementary molecular systematic data retrieved from full genome sequences. Subsequently, 10 additional *Frankia* species have been proposed for strains assigned

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to clusters 1 to 4, as exemplified by, *Frankia canadensis* (Normand et al., 2018), *Frankia torreyi* (Nouioui et al., 2018a), *Frankia coriariae* (Nouioui et al., 2017a), *Candidatus Frankia datiscae* (Persson et al., 2011), *Candidatus Frankia californiensis* (Normand et al., 2017), *Frankia discariae* (Nouioui et al., 2017b), *Frankia elaeagni* (Nouioui et al., 2016), *Frankia irregularis* (Nouioui et al., 2018b), *Frankia asymbiotica* (Nouioui et al., 2017c), *Frankia inefficax* (Nouioui et al., 2017d), and *Frankia saprophytica* (Nouioui et al., 2018c).

The marked improvements in the classification of the genus *Frankia* together with the availability of more than 35 genome sequences of representative strains provide an invaluable platform not only for promoting our understanding of actinorhizal symbionts, but also for exploitable biology. It is, for instance, particularly interesting that the genome of *Frankia* strains are rich in natural product biosynthetic gene clusters that encode for the biosynthesis of novel and uncharacterized specialized metabolites, some of which may prove to be of value in the search for new antibiotics needed to control multi-drug resistant microbial pathogens.

In addition, the availability of strains representing well described species open up the prospect of selecting appropriate strains for the reclamation of polluted (Richards et al., 2002; Rehan et al., 2014a,b; Baker et al., 2015) and saline soils (Tani and Sasakawa, 2003) and in raising the fertility of marginal land (Diagne et al., 2013) and as bioinoculants for enhancing the growth of specific host plants.

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Figure 1. Dr. Nisha Patel

Finally, we have some in-house news concerning the *Bulletin*. Since 2013, my graduate student, Nisha Patel (Fig 1), served as the Managing Editor. This summer after earning her PhD, she graduated and after a number of job offers took a position in Boston, MA with a Microbiome company. On behalf of BISMiS, I want to personally thank Dr. Nisha Patel for her tremendous efforts over the years and wish her well for her future in taking her taxonomic knowledge to our colleagues in industry. The *Bulletin* Managaing Editor position and duties have been assumed by one of my new graduate students, Shannon Fulton (Fig 2), and she has already performed a sterling job with this issue.

-Dr. Paul A. Lawson
Editor-in-Chief

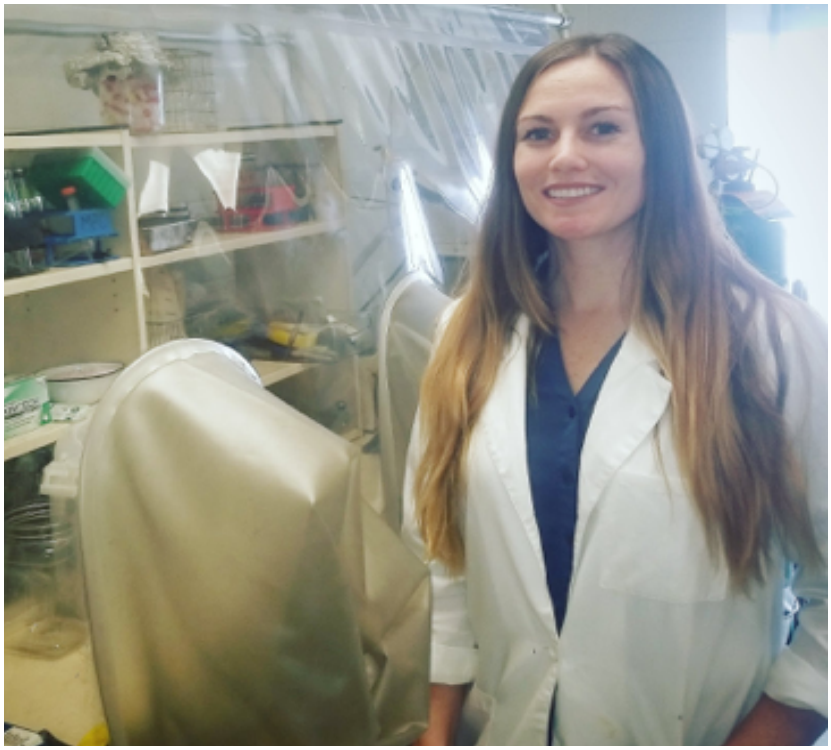

Figure 2. Shannon Fulton